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2nd International Conference on Neotropical Orchidology (ICNO)

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ARE OUR ORCHIDS SAFE?

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THE ORCHID CONSERVATION CHALLENGE

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In the six years since the first International Orchid Conservation Congress in 2001 has the conservation of orchids progressed? Certainly orchid science has grown from less than 100 published works in the 20 year period from 1900-1920 to over 3200 for 2000-2005. With knowledge of orchids spanning an astonishing array of disciplines it is therefore surprising that there remains a significant gap between orchid science and orchid conservation practice. This is no more telling than in the resolutions of the plenary session of the first IOCC to adopt for orchids four targets of the 16 targets from the Global Strategy for Plant Conservation (see http://www.bgci.org/worldwide/gspc/) – that by 2010 (just three years away….!) – 90% of threatened orchids are secure in ex situ conservation collections; 50% of these threatened orchids are in active recovery programs; no orchids are threatened by unsustainable harvesting; and, every child aware of plant diversity (including orchids). How have we tracked on delivering these four targets? Besides a growth in botanic gardens to almost 2500 worldwide, the proportion of orchids in ex situ conservation collections, particularly rare and threatened taxa, has barely changed in six years yet the science and technology to achieve this target is well established. Equally the pace of the development of orchid recovery plans is outstripped by the annual increase in orchid taxa being listed as rare. Indeed the most basic of information is often lacking in orchid conservation projects from knowledge of the causal factors of orchid rarity to whether research outcomes and management plans are being converted to successes in the field? An important criterion for recovery projects should be ' will it be possible to implement the results of the research – is the funding available and what are my cost-effective alternatives?' Some areas such as defining sustainability for the wild harvesting of orchids remains a complex and difficult issue often tied to local economy and cultural identity. The slow maturation of orchids means that any wild exploitation, unless carefully managed on scientific grounds, is bound to lead to a decline in the orchid. Finally, though the final target falls outside of the expertise of conservation scientists is in the long term, it is perhaps the most critical of all conservation actions for the long term conservation of orchids. It is much easier/ preferable/more fun to do research, write and field trip than to ensure that k-12 educational needs include sound (and fun!) conservation messages. Ultimately our ability to deliver effective conservation is controlled by funding bound to political processes that in themselves rely on awareness and education from an early age.

With the long term goal of greater community awareness and funding of conservation, as researchers we can maximize our conservation outcomes in the short term through the approach we take to research and the questions we ask. When attempting to conserve a particular species, establishing which aspect of the life cycle or human interaction is driving species rarity is a critical step. A key to success in orchid reintroduction is the need for integration of knowledge gaps – how many orchid reintroductions adopt a multidisciplinary approach? The majority of published works in orchid introductions rely on single principles as the basis for the reintroduction, often with a heavy emphasis on propagation/establishment techniques. However, pollination biology, mycorrhizal ecology, habitat requirements, changing habitat condition, habitat clearing and wild collecting are all potential causes of rarity that need to be considered for developing a multi-disciplinary and more sustainable conservation outcome for orchids.

An obvious division within orchid conservation biology is between the terrestrial and epiphytic life forms and the practical implications for conservation programs. For example, most terrestrial taxa have often

intricate and biologically sensitive interactions with mycorrhiza. The evidence for such relationships in epiphytes is less convincing – take for example the billions of orchid plants produced commercially each year in the vast production houses of Europe and south-east Asia all without specific fungal endophytes. Interestingly there are far fewer epiphytic orchid reintroduction programs compared with terrestrial orchids – possibly linked to the affluence of countries (mostly temperate climates that support only terrestrial orchid floras) and their ability to support reintroduction programs.

Beyond endophyte specificity/requirements, epiphytic and terrestrial orchids share much in common in their requirements for devising an effective conservation reintroduction or preservation program. Keystone knowledge needs to reflect the ability of the reintroduced or remaining populations of the plant to complete its life cycle successfully including self-perpetuating populations. Knowledge gaps that are critical for meeting this performance indicator include: understanding pollination syndromes, biology of the pollinator, substrate, successional requirements (successional vs climax vegetation), seed germination and seedlings establishment requirements and importantly the capacity of the species and the reintroduction to cope with climate change. The latter is one of the most significant challenges facing orchid conservation programs. Evidence is continuing to mount that for terrestrial orchids, range contractions or expansions may be a fact of life for many species just as the paleoecological data indicates such changes have occurred over the millenia for other plant groups.

Given the diversity of potential drivers of orchid rarity, future conservation programs will require researchers to draw information across a wide spectrum of scientific disciplines. However, this is important in integrating conservation of orchid species with the societies in which they occur. As one of the more sensitive components of the flora to environmental change, orchids can be justified as flagship species for plant conservation, both scientifically and through their widespread recognition in the wider community.

As some of the most charismatic of species for plant conservation, failure to deliver effective conservation of orchids, even using the simple approach of the GSPC orchid targets, represents a dire scenario for conservation of all plant species. Can we as orchid conservation professionals accept the challenge of delivering a more effective orchid conservation message to the wider world? Orchids continue to be the plants that captivate and enthrall. Movies are made about them ('Adaptation' with Nic Cage and Meryl Streep), we eat them (vanilla), they remain as symbols of love and devotion and they are the most recognised of plant families. As David Attenborough stated 'Orchids surely are the most glamorous of plants…'. The challenge therefore is to conclude the third IOCC with a renewed sense of purpose and direction melding as never before, science with orchid conservation practice. And by securing orchid conservation there is a very real opportunity for collateral conservation of a considerably larger biodiversity as orchids unlike most plant groups depend for their existence upon a host of other plants and animals. What better reason for conserving these remarkable plants.

Dr **Kingsley Dixon** has over 20 years experience in researching the ecology and physiology of Australian native plants and ecosystems. He leads a science group comprising botanical and restoration sciences and, as Director of Science at the Botanic Gardens and Parks Authority (BGPA), has developed a strong multi-disciplinary approach to conservation and restoration of native plant biodiversity and degraded landscapes. The research team of over 40 research staff and postgraduate students specialise in seed ecology and biology, propagation science, germplasm storage, conservation genetics and restoration ecology with a strong emphasis on orchid biology and conservation. This research group has contributed significantly to seed science in Australia, with major advances in understanding seed dormancy (pioneering work in smoke technology) as well as orchid seed conservation.

Ryan Phillips is a PhD student at Kings Park and Botanic Garden and the University of Western Australia working on the role of mycorrhiza and pollinators in controlling rarity and speciation in *Drakaea*. Interests include the causes of orchid diversification and rarity and the co-evolution of orchids and their pollinators.

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ORCHID CONSERVATION: WHERE DO WE GO FROM HERE?

PHILIP T. SEATON

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Introduction

All that remains of the dodo are a few relics scattered around the museums of the world, the odd pile of bones, the remnants of a head and a foot at the Oxford University Museum of Natural History. Is this to be the fate of many orchid species? An occasional dried plant, mummified between dusty herbarium sheets, or even worse, no record at all? Although there is little hard evidence that, as yet, many species have become extinct in the wild, the sizes of orchid populations are often declining alarmingly. On a recent Kew expedition to Madagascar for example, only 25 plants of *Angraecum longicalcar* were found remaining in the wild and, reputedly, only a single plant of *Grammangis spectabilis*, the remainder of this population having been collected from its natural habitat in 1993.

We are all too aware of the current threats to wild orchid populations, habitat destruction, land conversion, over-collection. Nor are such problems confined to tropical countries with their rich orchid floras, species are in decline in many temperate areas. As if the situation wasn't sufficiently alarming, new threats are looming on the horizon. There is evidence that global warming is already having adverse effects in cloud forests around the world, and the much-vaunted move towards a change to biofuels may, paradoxically have the effect of pushing agribusiness further into rainforests, and taking up even more valuable land.

Faced with such a multitude of threats to the natural world, it is all-too-easy to become despondent. Yet the orchid community in general is becoming much more aware of the problems and, increasingly prepared to take action. Thus, the purpose of this paper is to focus on what can and is being done to conserve orchids.

In situ conservation

In an ideal world, examples of all the world's different natural habitats would be preserved, and along with them all the orchid species they contain. Conservation of entire ecosystems should be our goal, but clearly this isn't going to happen in every case. Land purchase for reserves where possible, focusing on hotspots of biodiversity is, however, an important and effective strategy. The locations of orchid hotspots have been identified, and largely coincide with the biodiversity hotspots of other plant and animal groups. The conservation of the tropical dry forests of Costa Rica's Guanacaste province provide a shining example of what can be achieved with dedication and determination. Similarly El Pahuma and Jocotoco reserves in Ecuador have been established to conserve birds as well as orchids. Meadows, with their enormous plant diversity have been specifically targeted for purchase by the Wildlife Trusts in the UK, and my own home county contains many orchid-rich areas owned and managed by the Worcestershire Wildlife Trust.

Ex situ Conservation

There are those who express fears that if we spend our resources on *ex situ* conservation techniques this will reduce the impetus for conservation of habitats. *Ex situ* techniques should rather be seen as welcome additions to the conservation tool-box, essential components of an integrated approach.

Living Collections in botanical gardens have the capacity to perform both educational and research functions, as well as acting as an insurance policy against future losses. The collections of both commercial and amateur growers are equally important. Indeed, it was a commercial grower that provided us with the classic example of the potential importance of plant rescue and *ex situ* conservation. For many years, *Paphiopedilum delenatii* was thought to be extinct in the wild, and

was propagated in large numbers by Vacherot and Lecoufle. By producing good quality, affordable plants, commercial growers can reduce pressure from collection in the wild.

Amateurs have a positive role to play in orchid conservation. Often they are the best growers, having both the time and the dedication to nurture their plants. Many amateurs specialise in certain orchid groups, which in the UK can become recognised as National Collections. The Barbara Everard Trust was established in the UK to assist in resolving the problem of finding a suitable home for plants when the owner of the collection dies. The issue of long-term security of plants is not unique to amateur collections, however, botanical gardens may also encounter difficulties in maintaining continuity of care due to staff turnover.

An orchid collection should be viewed as being a dynamic entity. However long or short-lived individual plants may be, there will be a constant and on-going need to regenerate plants. There is a strong case to be made for the establishment of a network of living collections, first identifying the species which are rare in cultivation, then propagating and sharing them, including a scheme to maintain genetic diversity by out-crossing.

Techniques for growing orchids seldom get much of an airing, if they get an airing at all, at orchid conservation events. Perhaps they are viewed as being the province of the horticultural world. And yet there is a need for sharing best-practice regarding the cultivation of rare species in particular. In this respect (and others) the American Orchid Society (AOS) web site provides a valuable service. Pest and disease control are essential components of maintaining healthy plants in virus-free collections.

Growing Orchids from Seed

With increasing regulation it is becoming ever more difficult for the amateur grower, in temperate countries at least, to obtain species for cultivation in collections.. Yet the production of plants from seed is not difficult, and reduces the pressure on wild populations from unscrupulous collectors. With the increasing availability of pre-packaged media and simple step-by-step manuals, growing orchids from seed does not need sophisticated laboratory equipment, and is relatively straightforward to perform in the home.

Often the only limitation is obtaining good, viable orchid seed. This has been resolved, at least in part, by the setting up of short-term seed banks in many countries. Seed can, for example, be purchased from the Orchid Seedbank Project (OSP) in the USA. Long-term storage requires a greater financial investment. Larger institutions such as The Royal Botanic Gardens, Kew's Millennium Seed Bank can provide a means of preserving maximum genetic diversity in a minimum space and at a minimum cost.

The development of techniques for germination, particularly for some of the more 'difficult' temperate species has raised the possibility of habitat restoration and re-introduction, exemplified by the pioneering Sainsbury Orchid Conservation Project to reintroduce *Cypripedium calceolus* in the UK, which at one time had declined to just one individual plant in the wild due to over collection.

Sustainable development

The collection and sale of wild plants seems to have become something of a taboo subject, and yet there could be a place for the collection and sale of some orchids if it is done in a controlled and renewable fashion. More often than not, there is a lack of data about the status of wild plants. *Cattleya dowiana* is still being collected from the forests of Turrialba in Costa Rica for example and yet, in part due to the difficulties of access, no studies are in place to study the impact, if any, of its continued removal.

The idea of sustainable development is viewed by some as being something of a contradiction in terms, what is clear, however, is that involvement of local people is key to the success of many projects. Many *Laelia* species in Mexico are traditionally used as cut flowers for use in religious festivals. Local people could be encouraged to cultivate plants generated by *in vitro* propagation techniques, rather than collecting plants from the wild. A project at Soconusco directed by Anne Damon is encouraging campesinos to cultivate their local

orchids sustainably. I am not suggesting for a moment that such projects can provide would be a sole source of income, rather they could form part of a mixed portfolio. Likewise the encouragement of ecotourism can provide an income for a range of associated businesses, such as Gabriel Barboza's orchid garden on the margins of the Monteverde Cloudforest Reserve.

Many tropical countries have built or are in the process of building their own indigenous orchid industries. Over recent years there has been an explosion in the production and sale of *Phalaenopsis* as pot plants for example. There are also opportunities to establish smaller nurseries catering for the needs of specialist orchid growers.

Education

It is encouraging to see more articles with a conservation focus appearing in orchid journals such as *The Orchid Review* and *Orchids*, and there does appear to be an increasing interest in environmental issues by the general public, if only because of rapidly increasing concerns about the effects of global warming. Although not able to compete with the so-called 'charismatic megafauna', orchids have been referred to as the 'pandas of the plant world'. There is no doubt that they have a special aura and could act as flagships for plant conservation in general.

The need to involve young people is self-evident, indeed our efforts are largely devoted to preserving the orchid diversity for them to enjoy. The project at Writhlington School in the UK serves as a beacon, where young people are encouraged to participate in a wide range of orchid activities ranging from growing and showing orchid to visits to tropical climes to carry out conservation projects. It has already spawned at least one imitator at King Charles 1 school in Kidderminster in a project aimed at raising local orchids to set up an orchid garden at the headquarters of the local Wildlife Trust.

As part of a strategy to engage a wider audience as possible Orchid Conservation International has set up a partnership with staff and students at Blackpool and The Fylde College in the UK. Students who are studying for a degree in Scientific and Natural History Illustration have produced two posters. Not only are these posters attractive in their own right, by telling different stories they can act as valuable teaching tools. The first, by Ian Cartwright, tells the story of the bucket orchid, *Coryanthes kaiseriana* and its euglossine bee pollinator, the second of *Paphiopedilum rothschildianum* on the slopes of Borneo's Mount Kinabalu, reduced to a handful of plants in remnant populations.

NGO's, Orchid Charities and Sources of Funding

From the above it is clear that there are a wealth of ways in which orchid conservation can be promoted, and of orchid conservation projects worth funding. Obtaining funding is of course a perennial problem. The Orchid Specialist Group lists possible sources on its website.

In addition to the long-standing commitment of the AOS, a number of orchid charities have been established recent years, including Orchid Conservation International (OCI), Orchid Conservation Alliance (OCA) and the Orchid Conservation Coalition (OCC) - 1% for orchid conservation. The specific aims may vary according to the organisation, but all aim to support a broad range of orchid conservation activities in one way or another. For instance, in addition to supporting the work of the Orchid Specialist Group (OSG), OCI has recently awarded grants both for an educational orchid programme and seed money for a field project conducted by WildShare at the El Cielo Biosphere Reserve in Mexico, and for a project monitoring epiphytic orchid distribution and population dynamics on a disturbance gradient in Andean cloud forests in Colombia.

Nor should it be forgotten that gifts in kind are always welcome from private individuals. Donations of microscopes and books may appear to be obvious, but also equipment such as scanners for scanning herbarium sheets. Of course orchid charities are themselves constantly searching for new sources of funding to allow them to continue their work.

Finally, the Orchid Specialist Group is itself a volunteer organisation. Made up of around two

hundred individuals with a wide range of expertise; botanists and other scientists, commercial growers and amateurs, it has an important educational role through its promotion of International Orchid Conservation Cogresses, its web site and its on-line journal *Orchid Conservation News*. Together with the provision of advice and through the mobilisation of its membership it has the potential to have a large and positive impact on orchid conservation over the coming years.

Previously a biology lecturer, **Philip Seaton** now devotes himself full-time to orchid conservation. He is Secretary of Orchid Conservation International and the Orchid Specialist group, and runs a micropropagation laboratory at a local school. A past editor of *The Orchid Review*, he has written around one hundred popular and scientific articles on a wide range of orchid topics. He has illustrated and co-authored *Growing Orchids from Seed*. He received the degree of Master of Philosophy for his research into orchid seed storage, and is currently working to promote the establishment of a global network of orchid seed banks.

GEOGRAPHY OF CONSERVATION

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INVASIVE ORCHIDS: WEEDS WE HATE TO LOVE?

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Rare species that show habitat specificity and an aversion to habitat disturbance may be common in the Orchidaceae (Tremblay et al. 1998; Bergman et al. 2006). Nonetheless, most orchids may not be in such a critical state and many are, quite frankly, weedy. We may learn much about rare species by asking what makes other orchids common and resilient or actually dependent on change. Most orchids do occur in ephemeral or frequently disturbed habitats (Ackerman 1983; Catling 1996) whether they are pastures, roadsides, citrus groves, coffee and tea farms, or simply as epiphytes whose substrates, by definition, are temporary and run the gamut from durable tree trunks to short-lived twigs (Johansson 1974).

Effective dispersal capabilities are essential for occupying ephemeral habitats. Certainly orchid seed morphology lends itself to the possibility of distance dispersal (Arditti & Ghani 2000; Murren & Ellison 1998). In the West Indies, nearly 60% of the orchid species occur on more than one island and a floristic affinity analysis has indicated that geographical distance for these species is generally not a barrier to dispersal (Trejo-Torres & Ackerman 2001).

The combination of mobility and widespread preference for ephemeral habitats appears to have given orchid populations a degree of resiliency that is generally underappreciated. We all know that deforestation, or habitat destruction is a common problem not only in the tropics but elsewhere throughout the world. A prime example is Puerto Rico where 95% of the forest cover was cut by the early 1940's (Brash 1987; Lugo et al. 1993 cited by Figueroa Colón 1996), yet the number of orchid species lost from the flora has been less than 5%. Since then, forest recovery has been substantial and some orchid species have responded positively to the re-growth, a few becoming quite abundant in secondary habitats (Ackerman & Galarza-Pérez 1991).

Orchids with rapidly expanding populations include natives, but non-natives everywhere are making their appearance (Table 1). In fact, the global compendium of weeds (www.hear.org/gcw/index.html) lists over 90 orchid weeds! In Puerto Rico, a number of non-native orchids have persisted for a long time, but only recently have they become aggressive taking on weed-like characteristics. Such a demographic pattern is very typical of invasive species.

What makes an orchid weedy and invasive? Many plants that are classified as weeds have a suite of characteristics associated with colonization capabilities, and some of these features characterize orchids in general: abundant seed production (although in orchids effective population sizes may be small), distance dispersal, and weak competitive capabilities. Rapid development, autogamy and apomixis are also common features of weeds, but these are certainly not common features of orchids. From a sample of weedy orchids, we find a complete spectrum of breeding systems (e.g., Sun 1997), from apomictic or autogamous to outcrossing, and plants of the latter may be either self-compatible or -incompatible.

It is difficult to find a common thread among the invasive orchids. Some are understory plants; perhaps most prefer grassy roadsides, while a few others are epiphytes. Some are autogamous but others attract local pollinators with nectar rewards or by deceit, with pollination systems not unlike that of local species. Answers may rest not only with the distribution of appropriate habitat, but also with the

TABLE 1. Orchid species naturalized in Puerto Rico.

Species	**Native**	**Non-native**	**Breeding** system	**Habitat**
Arundina graminifolia	India, Nepal, China, SE Asia to Indonesia	Hawaii, Puerto Rico, Guadeloupe	Outcrossing Deception	Open disturbed
Dendrobium crumenatum	India, Bangladesh, China, Burma, Thailand, Vietnam, Indonesia, Malaysia, Philippines, Australia	Puerto Rico, Guadeloupe	Outcrossing reward	Open
Epidendrum radicans	Mexico, Central America, Colombia	Cuba, Puerto Rico	Outcrossing deception	Open disturbed
Oeceoclades maculata	Africa	South America, Central America, West Indies, Florida	Autogamous	Shady
Phaius tancarvilleae	India, Nepal, China, SE Asia to Phillipines, South Pacific Islands	Hawaii, Cuba, Jamaica, Puerto Rico	Outcrossing? Deception	Open to shady disturbed
Spathoglottis plicata	India, SE Asia New Guinea, New Caledonia to Phillipines	Hawaii, Cuba, Puerto Rico, Virgin Islands, Lesser Antilles	Autogamous	Open, disturbed ground
Vanilla planifolia	Mexico, Central America?	Puerto Rico, West Indies, Central & South America	Outcrossing deception*	Forest disturbed habitats
Vanilla pompona	Mexico, Central America, South America	Puerto Rico, Lesser Antilles?	Outcrossing deception*	Forest disturbed habitats
Zeuxine strautematica	Sri Lanka, India, SE Asia, Java, Phillipines, Taiwan, Japan	Southern USA, Bahamas, Cuba, Jamaica, Puerto Rico	Apomictic	Open disturbed

*The two vanillas may be reward plants at least for the male euglossine bee pollinators in Central America.

players in the orchids' symbiotic relationships: pollinators and mycorrhizal fungi. Widespread species either specialize on widespread "partners" or are catholic with whom they play or exploit (cf. Bascompte et al. 2003; Vázquez & Aizen 2004). The asymmetrical relationship between plants and their pollinators is well documented, but the relationship between orchids and their mycorrhizal symbionts is only just beginning to be revealed (e.g., Otero *et al.* 2002, 2004; Taylor *et al.* 2003). What do rare species do? Again, we do not know this entirely but we can predict that constraints of specificity may have a role, whether it is the habitat, the pollinators, their mycorrhizal associations, or some combination of the three remains to be seen.

Finally, we are faced with an orchidaceous dilemma: can non-native, marquee taxa be so bad? Do they exclude native taxa or disrupt natural ecosystems or are they benign? Do we encourage, tolerate or fight such intrusions to our sovereign soil?

LITERATURE CITED

Ackerman, J.D. 1983. On the evidence for a primitively epiphytic habit in orchids. Syst. Bot. 8: 474-477.

Ackerman, J.D., J.C. Trejo Torres & Y. Crespo Chuy. In press. Orchids of the West Indies: predictability of diversity and endemism. J. Biogeography.

Ackerman, J.D. & M. Galarza-Pérez. 1991. Patterns and maintenance of extraordinary variation in the Caribbean orchid, *Tolumnia* (*Oncidium*) *variegata.* Syst. Bot. 16: 182-194.

Arditti, J. & A.K.A. Ghani. 2000. Numerical and physical properties of orchid seeds and their biological

implications. New Phytol. 145: 367-421.

Bascompte, J., P. Jordano, C.J. Melián & J.M. Olesen. 2003. The nested assembly of plant-animal mutualistic networks. Proc. Nat. Acad. Sc. (USA) 100: 9383-9387.

Bergman, E., J.D. Ackerman, J. Thompson & J.K. Zimmerman. 2006. Land use history affects the distribution of the saprophytic orchid *Wullschlaegelia calcarata* in Puerto Rico. Biotropica 38: 492-499.

Brash, A. 1987. The history of avian extinction and forest conversion on Puerto Rico. Biol. Conserv. 39: 97-111.

Catling, P.M. 1996. Conservation strategy. Pages 11-23 *in*: IUCN/SSC Orchid Specialist Group, Orchids—status survey and conservation action plan. IUCN Gland, Switzerland and Cambridge, UK.

Figueroa Colón, J.C. 1996. Phytogeographical trends, centers of high species richness and endemism, and the question of extinctions in the native flora of Puerto Rico. Ann. New York Acad. Sc. 776: 89-102.

Johansson, D. 1974. Ecology of West African epiphytes. Acta Phytogeog. Suec. 59: 1-129.

IUCN/SSC Orchid Specialist Group. 1996. Orchids—status survey and conservation action plan. IUCN Gland, Switzerland and Cambridge, UK.

Lugo, A., J. Parrotta & S. Brown. 1993. Loss in species caused by deforestation and their recovery through management. Ambio 22(2-3): 106-109.

Murren, J.C. & A.M. Ellison. 1998. Seed dispersal characteristics of *Brassavola nodosa* (Orchidaceae). Amer. J. Bot. 85: 675-680.

Otero J.T., J.D. Ackerman& P. Bayman. 2002. Diversity and host specificity of endophytic *Rhizoctonia*-like fungi from tropical orchids. Amer. J. Bot. 89: 1852-1858.

Otero J.T., J. D. Ackerman & P. Bayman. 2004. Differences in mycorrhizal preferences between two tropical orchids. Molec. Ecol. 13: 2393–2404.

Sun, M. 1997. Genetic diversity in three colonizing orchids with contrasting mating systems. Amer. J. Bot. 84: 224-232.

Taylor, D.L., T.D. Bruns, T.M. Szaro & S.A. Hodges. 2003. Divergence in mycorrhizal specialization within *Hexalectris spicata* (Orchidaceae), a nonphotosynthetic desert orchid. Amer. J. Bot. 90: 1168-1179.

Trejo-Torres, J.C. & J.D. Ackerman. 2001. Biogeographic affinities of Caribbean Orchidaceae based on parsimony analyses of shared species. J. Biogeogr. 28: 775-794.

Tremblay, R.L., J.K. Zimmerman, L. Lebrón, P. Bayman, I. Sastre, F. Axelrod & J. Alers-García. 1998. Host specificity and low reproductive success in the rare endemic Puerto Rican orchid *Lepanthes caritensis* (Orchidaceae). Biol. Conserv. 85: 297-304.

Vázquez, D.P. & M.A. Aizen. 2004. Asymmetric specialization: a pervasive feature of plant-pollinator interactions. Ecology 85: 1251-1257.

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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF SPECIES OF *TULASNELLA* (HOMOBASIDIOMYCETES) ASSOCIATED WITH NEOTROPICAL PLANTS OF LAELIINAE (ORCHIDACEAE) OCCURING IN BRAZIL

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Introduction

Tullasnella spp. have been found forming mycorhizal associations with plants of all Orchidaceae subfamilies, and they are one of the main symbionts in partially micoheterotrophic plants (Taylor *et al.* 2002). Little is known about mycorhizal fungi of Neotropical Orchidaceae, especially in Laeliinae that occur in distinct environments such as "Restingas", Seasonal Forests and "Campos Rupestres" (Cruz *et al.* 2003, Britto *et al.* 1993, França *et al.* 1997, Withner 2000).

Some few studies in completely mycoheterotrophic Epidendroideae have been shown that these plants form mycorrhizal associations mainly with fungi of the genera *Russula*, *Thelephora*, *Sebacina*, as well as other ectomycorrhizal Basidiomycetes in trees (Taylor and Bruns, 1999, 1997, Taylor *et al.* 2003, Selosse *et al.* 2002, Girlanda *et al.* 2006). There are other studies indicating a preferential association between basidiomycetous fungi and Orchidaceae plants as in Oncidiinae with *Ceratobasidium* and *Cypripedium* with *Tulasnella* (Otero *et al.* 2002, 2004, Shefferson *et al.* 2005). These works suggest a putative specificity and recruiting of these plants in the environment where they occur.

Laeliinae plants have been intensively and indiscriminately collected in Brazil, leading to a significant reduction in their natural populations. In order to establish conservation strategies to these threatened plants as there is an indication in literature showing a preferential association between some specific fungi and Orchidaceae, the identity of symbiont fungi forming mycorrhizal associations in Brazilian Laeliineae was studied, aiming to an efficient *in situ* and *ex situ* conservation.

Methodology

COLLECTION SITES AND ISOLATION OF FUNGI

Orchidaceae plants were collected from natural populations that occur in two distinct Brazilian States. A total of 20 natural populations, including plants of Laeliinae and Pleurothallidinae were sampled. From each population, one or two individual plants were collected and their roots were sampled in a period of one to two weeks since collection date. The individuals were selected from distinct environments (Tropical Rain Forest, "Restinga", and "Campo Rupestre") and the isolation of associated fungi was carried out according to Warcup and Talbot (1967).

MORPHOLOGICAL CHARACTERIZATION OF FUNGAL COLONIES

Fungal colonies were incubated for 30 days in PDA (potato-dextrose agar) and OA (3% oat meal agar) to induce the formation of monilioid cells, and they

were further analysed to determine the form, number and array of the cells. Macroscopic and microscopic somatic features of the colonies were also described. In order to analyse the nuclear condition, hyphal nuclei were stained according to Sneh *et al.* (1991).

MOLECULAR CHARACTERIZATION OF FUNGAL ISOLATES

All the isolates were first cultivated in BDA for 15 days at 28 °C , including an *Epulorhiza epiphytica* Pereira, Rollemberg et Kasuya isolate, gently sent by Mycorrhizal Association Lab of the Federal University of Viçosa, Brazil. DNA extraction was carried out according to CTAB protocol (Doyle & Doyle, 1987). Double-stranded symmetric PCR reactions were carried out in 0.2-mL tubes in 50 µL reaction volume, using the primers ITS5 and ITS4 that amplify the Internal Transcribed Spacer (ITS region) of nuclear ribosomal DNA (White *et al*., 1990). PCR products were purified using EXOSAP and were sequenced in an automatic DNA sequencer (SCE 2410, Spectrumedix LLC). Chromatograms were edited using GAP4 software in Staden (Staden, 1996). Resulting sequences were submitted to a similarity search using BLASTn software of NCBI and aligned with Clustal X (Thompson *et al.* 1997). Phylogenetic parsimony analyses (heuristic search, TBR algorithm) were conducted in PAUP 4.0 (Swofford, 1998). Clade robustness was assessed using bootstrap proportions (1000 replications) (Felsenstein, 1985).

Results and discussion

IDENTIFICATION OF ISOLATES FROM LAELIINAE

According to morphological characterization, the isolates belong to the genus *Tulasnella* (Basidiomycetes) (Rasmussen 1995, Currah and Zelmer 1992, Currah *et al.* 1997b), but the somatic characters were not stable enough to differentiate the groups. All the colonies presented an entire submersed margin and binucleate hyphae (Fig.1). In all the isolates monilioid cells showed a very high morphological plasticity with cell chains ranging from 3 to 15 cells with or without ramification. Andersen (1990) pointed out that somatic features were not reliable, since there is not even one character that could be taken as a parameter in intraspecific level. The three isolates showed a growing pattern typical of rhizoctonoid fungi, but they did not produce monilioid cells even when they were submitted to distinct culture media.

All the sequences were compared to NCBI database, revealing that the isolates belonged to different lineages of *Tulasnella* including *T. violea* and *T. calospora*. Some sequences were considerably difficult to align and they were initially excluded from the phylogeny. In the phylogenetic tree (Fig. 2) some of the isolates represented lineages of *Tulasnella calospora* and others were lineages of *Epulorhiza epiphytica*, both of them significantly supported by bootstrap analysis. *E. epiphytica* is the only species described for Brazil and it was isolated from host plants that naturally occur in the State of Minas Gerais (Pereira *et al.* 2003). These results suggest that all the isolates are distinct lineages of *Tulasnella*, and that this possibly reflects the different environments where host plants occur.

RELATIONSHIPS BETWEEN LAELIINAE AND TULASNELACEAE

In accordance to the results, although host plants live in completely different environments where the research availability is distinct, one can observe the strong trend of studied plants to form mycorrhizal associations with fungi of the genus *Tulasnella* (Almeida 2006). Studies on Australian orchids revealed that Diurideae plants has a strict specificity relationship with the fungi *Sebacina vermifera* and some lineages of *Tulasnella*, including *Tulasnella calospora*, which has been considered as a universal species (Rasmussen, 1995, Warcup, 1981, 1988, 1971). Inside Diurideae, all the studied species that belong to Drakaeinae and Diuridinae associate to *Tulasnella*, and all the studied species (except for those from genera *Lyperanthus* and *Bumettia*) that belong to Caladeniinae present a strict relationship with *Sebacina* (Warcup, 1981, Dressler, 1993). As all the isolates were obtained from *pelotons*, they are mycorrizal fungi.

Despite of the great advances obtained with the direct identification of fungi by molecular techniques such as PCR and sequencing, the morphological study of the isolates is still very important, mainly for the establishing of true biological entities or species.

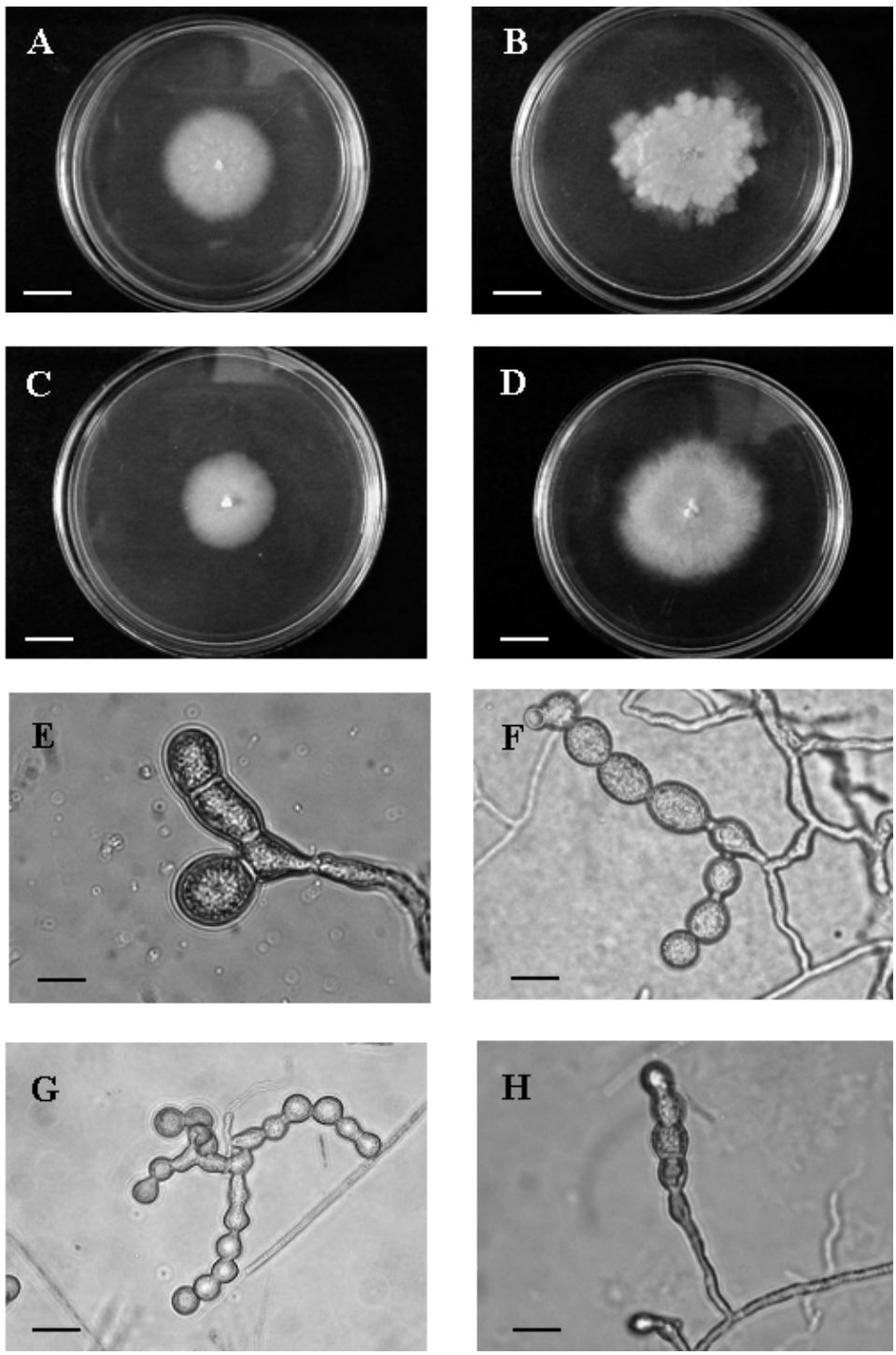

FIGURE 1. Any isolates of plants of Laeliinae. **A.** Isolate of *Acianthera hamosa*. **B.** *Cattleya elongata*. **C.** *Brassavola tuberculata*. **D.** *Dimerandra emarginata*. Scale bar is 1 cm. Any monilioid cells of other isolates. **E.** Isolate of *Sophronitis flavasulina*. **F.** *Sophronitis pabstii*. Scale bar is 3 ?m, **G.** *Epidendrum orchidiflorum* and **H**. *Cattleya tenuis.* Scale bar 5 ?m.

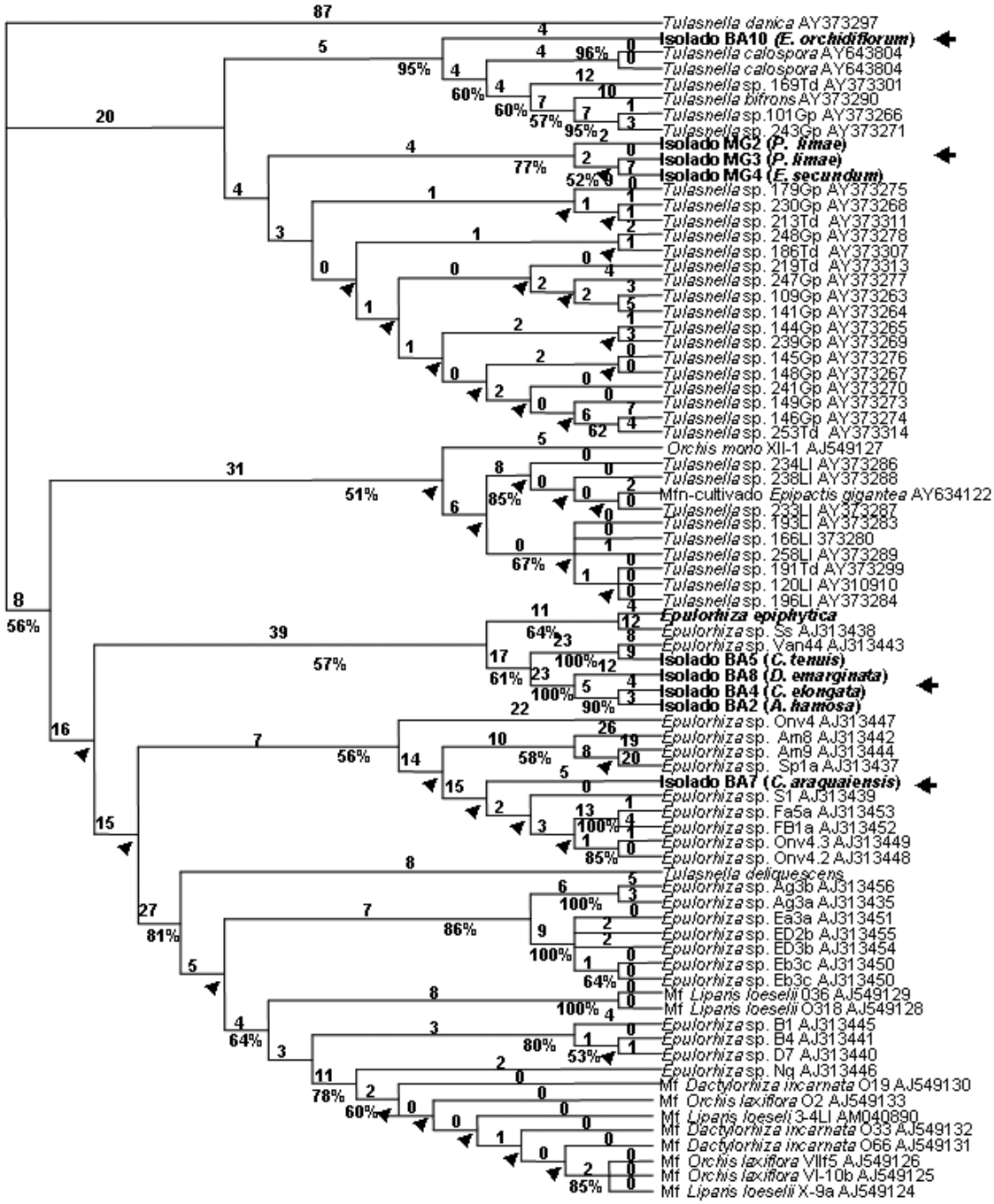

FIGURE 2**.** Fungal internal transcribed spacer phylogeny suggesting that the isolates of Laeliinae form mycorrhizal associations with fungi of the genus *Tulasnella*. The arrows show where the isolates of Laeliinae are.

Currently these studies have been decreasing, which reflects, for instance, the insignificant number of anamorphic fungi of described *Epulorhiza* species (Currah and Zelmer, 1992, Zelmer and Currah, 1995, Currah *et al.* 1997a, Pereira *et al.* 2003), as well as the high number of sequences deposited in GenBank without any definition in the specific level (McCormick *et al.* 2004, Shefferson *et al.* 2005).

It is not known if this putative preference could be extended to all genera inside Laeliinae. Some studies has already pointed out this possible preferential relationship in the mycorrhizal association in some few species of Laeliinae (Curtis, 1939, Nogueira *et al.* 2005, Pereira *et al.* 2001, 2003, Zettler *et al.* 1999). Future investigations will be carried out in order to verify the pattern of mycorrhizal association in Laeliinae genera for the development of a future program of symbiotic propagation of threatened Brazilian species.

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LITERATURE CITED

Almeida, P.R.M.de 2006. Associação micorrízica na subtribo Laeliinae (Orchidaceae). Monograph. State University de Feira de Santana, 56 p.

Andersen, T.F. 1990. A study of hyfal morphology in the form genus *Rhizoctonia*. Mycotaxon 37: 25-46.

Britto, I.C., L.P. Queiroz, M.L.S. Guedes, N.C. Oliveira & L.B. Silva. 1993. Flora Fanerogâmica das dunas e lagoas do Abaeté, Salvador, Bahia. Sitientibus 11: 31-46.

Cruz, D.T. da, E.L. Borba & C. van Den Berg. 2003. O gênero *Cattleya* Lindl. (Orchidaceae) no estado da Bahia, Brasil. Stientibus 3 (1/2): 26-34.

Currah, R.S. & C.D.A. Zelmer. 1992. Key and Notes for the Genera of Fungi with Orchids and a new Species in the Genus *Epulorhiza*. Rep. Tottori Mycol. Inst. 30: 43-59.

Currah, R.S., L.W. Zettler & T.M. McInnis. 1997a. *Epulorhiza inquilina* sp. nov. from *Platanthera* (Orchidaceae) and a Key to *Epulorhiza* Species. Mycotaxon 61: 338-342.

Currah, R.S., C.D. Zelmer, S. Hambleton, & K.A. Richardson. 1997b. Fungi from orchid mycorrhizas. In: Arditti, J. & Pridgeon, A. M. Orchid Biology. Kluwer Academic Publishers. Dordrecht/Boston/London, 1997, 117-170.

Curtis, J.T. 1939. The relation of specificity of orchid mycorrhizal fungi to the problem of symbiosis. Am. J. Bot. 26: 390-398.

Doyle, J.J. & J.L. Doyle. 1987. A rapid isolation procedure for small quantities of fresh tissue. Phytochemical Bulletin, 19: 11-15.

Dressler, R.L. 1993. Phylogeny and classification of the orchid family. Dioscorides Press, Portland.

França, F., E. Mello & C.C. Santos. 1997. Flora de *Inselbergs* da região de Milagres, Bahia, Brasil: I. Caracterização da vegetação e lista de espécies de dois *Inselbergs*. Sitientibus 17: 163-184.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evol. 39: 783-91.

McCormick, M.K., D.F. Whigham & J. O'Neill. 2004. Mycorrhizal diversity in photosynthetic terrestrial orchids. New Phytol. 163: 425-438.

Nogueira, R.E., O.L. Pereira, C.M. Kasuya, M.C. Lanna da S. & M.P. Mendonça. 2005. Fungos micorrízicos associados a orquídeas em campos rupestres na região do Quadrilátero Ferrífero, MG, Brasil. Acta Bot. Bras. v.19, 3: 417-424.

Otero, J.T., J.D. Ackerman & P. Bayman. 2002. Diversity and Host Specificity of Endophytic *Rhizoctonia* – like Fungi from Tropical Orchids. Am. J. Bot. v. 89, 11: 1852-1858.

Otero, J.T., J.D. Ackerman & P. Bayman. 2004. Differences in mycorrhizal preferences between two tropical orchids. Mol. Ecol. 13: 2393-2404.

Pereira, L.O. 2001. Caracterização morfológica e molecular de fungos micorrízicos de sete espécies de orquídeas neotropicais. 47 pg. Dissertação (Mestrado em Microbiologia do Solo), Universidade Federal de Viçosa, UFV, Minas Gerais.

Pereira, L.O., C.L. Rollemberg, A.C. Borges, K. Matsouka & M.C.M. Kasuya. 2003. *Epulorhiza epiphytica* sp. nov. isolada from mycorrhizal roots of epiphytic orchids in Brazil. Mycoscience, 44: 153-155.

Rasmussen, H.H. 1995. Terrestrial Orchids from seed to Mycotrophic plant. Cambridge University Press.

Swofford, D.L. 1998. PAUP: Phylogenetic analysis using parsimony and other methods, version 4.0 b6. Sunderland: Sinquer.

Shefferson, R.P., M. Weib, T. Kull & L. Taylor. 2005. High specificity generally characterizes mycorrhizal association in rare Lady's Slipper orchids, genus *Cypripedium*. Mol. Ecol. 14: 613-626.

Sneh, B., L. Burpee, A. Ogoshi. 1991. Identification of *Rhizoctonia* species. APS Press, The American Phytopathological Society, St. Paul, Minnesota, USA.

Taylor D.L., T.D. Bruns. 1997. Independent, specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids. Proc. Nat. Acad. Sci. USA 94: 4510-4515.

Taylor, D.L. & T.D. Bruns. 1999. Population, habitat and genetic correlates of mycorrhizal specialization in the 'cheating' orchids *Corallorhiza maculata* and *C. mertensiana*. Mol. Ecol. 8: 1719 – 1732.

Taylor, D.L., T.D. Bruns, J.R. Leake & D.J. Read. 2002. Mycorrhizal specificity and function in mycoheterotrophic plants. In: van der Heijden, M. C. A., Sanders I, eds. Mycorrhizal Ecology. Berlin, Germany: Springer – Verlag, p. 375-413.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin & D.G. Higgins. 1997. The clustral – Windows interface: flexible strategies for multiple sequence alingnment aided by quality analysis tool. Nucl. Acids Res. 24: 4876-4882.

Wacurp, J. H. & P.H.B. Talbot. 1967. Perfect states of rhizoctonias associated with orchids. New Phytol. 66: 631-641.

Wacurp, J.H. & P.H.B. Talbot. 1971. Perfect states of rhizoctonias associated with orchids - II. New Phytol. 70: 35-40.

Wacurp, J.H. 1981. The mycorrhizal relationship of Australian orchids. New Phytol. 87: 371-381.

Wacurp, J.H. 1988. Mycorrhizal associations of isolates of *Sebacina vermifera*. New Phytol. 110: 227-231.

Withner, C.L. 2000. The Cattleyas and their relatives. Vol. VI. The South American *Encyclia* Species. Timber Press, Portland, 153 p.

White, T.J., T.D. Bruns, S. Lee & J.W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 *in*: G. Maidh, J.J. Sninsky & T.J. White (eds.), PCR Protocols: A guide to methods and applications. New York: Academic Press.

Zelmer, C.D. & R.S. Currah. 1995. Ceratorhiza pernacatena and Epulorhiza caledulina spp. nov.: mycorrhizal fungi of terrestrial orchids. Can. J. Bot. 73: 1981-1985.

Zettler, L.W., Burkhead, J.C., Marshall, J.A. 1999. Use of a mycorrhizal fungus from *Epidendrum conopseum* to germinate seed of *Encyclia tampesis in vitro*. Lindleyana 14: 102-105.

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ARE OUR ORCHIDS SAFE DOWN UNDER? A NATIONAL ASSESSMENT OF THREATENED ORCHIDS IN AUSTRALIA

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Introduction

Australia has about 1700 species of orchids, comprising about 1300 named species in about 190 genera, plus at least 400 undescribed species (Jones 2006, pers. comm.). About 1400 species (82%) are geophytes, almost all deciduous, seasonal species, while 300 species (18%) are evergreen epiphytes and/or lithophytes. At least 95% of this orchid flora is endemic to Australia. While the tropical and subtropical epiphytic/lithophytic orchid flora is low by world standards, the temperate terrestrial orchid flora is amongst the richest and most diverse of any comparable region in the world.

Like many places on our planet, biodiversity and natural habitats in Australia have suffered substantial declines and range in abundance through agricultural, industrial and urban development. The Australian Government Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) currently lists 106 species of flora and fauna as extinct, and a further 1582 species as threatened, in Australia. Many orchid species are included in this list. This paper examines the listing process for threatened orchids in Australia, compares regional and national lists of threatened orchids, and provides recommendations for improving the process of listing regionally and nationally threatened orchids.

Methods

The national government of Australia and each of the six Australian state and two territory governments have processes for listing threatened species under biodiversity conservation legislation within each jurisdiction (Table 1). To establish the number of threatened orchids included in these lists, the schedules of each Act were checked and listed orchids identified (Appendix 1). The State of Victoria also maintains a published 'advisory' (non-legislative) list of rare or threatened flora. This list was also checked for numbers of threatened orchids and compared against the state legislative list. Comprehensive reviews of the conservation status of orchids at the

TABLE 1. National and state/territory legislation listing extinct, rare or threatened orchids in Australia.

Jurisdiction	Legislation
Australia	Environment Protection & Biodiversity Conservation Act 1998
Australian Capital Territory	Nature Conservation Act 1980
New South Wales	Threatened Species Conservation Act 1995
Northern Territory	Parks & Wildlife Conservation Act 2000
Queensland	Nature Conservation Act 1992
South Australia	National Parks & Wildlife Act 1972
Tasmania	Threatened Species Protection Act 1995
Victoria	Flora & Fauna Guarantee Act 1988
Western Australia	Wildlife Conservation Act 1950

state level, for South Australia (Bates 2006), and Victoria (Backhouse & Cameron 2005, DSE 2005), were checked and compared to official legislative lists for those states.

Scientific names were generally left as they were on the lists, despite many names no longer being valid due to changes in taxonomy. For instance, all Australian species of *Bulbophyllum* Thouars and all but one Australian *Dendrobium* Sw. species have been assigned to new genera (Jones 2006). However, in a few cases, species were listed under different names on different lists eg. the current name *Corunatylis tecta* (D.L. Jones) D.L. Jones & M.A. Clem. is listed under the EPBC Act, while the former name *Genoplesium tectum* D.L. Jones is still listed under the Queensland *Nature Conservation Act* 1992. In these cases, the currently accepted scientific name has been used.

Definitions – Any examination of different systems for describing the conservation status of threatened species immediately runs into the issue of varying classification systems and definitions. The nine state/territory and national legislative systems collectively use eight terms to describe conservation status (Table 2). In this paper, the following definitions are used:

- Threatened includes 'critically endangered', 'endangered' and 'vulnerable' species (*sensu* IUCN 2001). Note that 'rare' is not generally included in the definition of 'threatened'.
- Conservation Concern includes all 'extinct', 'threatened', 'rare', 'insufficiently known' or 'data deficient' species (*sensu* Backhouse & Cameron 2005).
- Listing is used to describe the formal process of adding a species to a threatened species list (usually called a Schedule) in biodiversity conservation legislation (Act).

Results

NATIONAL ASSESSMENT – A total of 424 orchid species are listed as extinct, threatened or rare in Australia (Appendix 1, summarised in Table 2). This total includes 195 species (about 12% of the Australian orchid flora) listed as extinct or threatened nationally, plus an additional 238 species (about 14% of the Australian orchid flora) listed as extinct, threatened or rare at the regional (ie. state or territory) level. State and territory threatened species lists include another 52 species that are listed as extinct or threatened within their jurisdiction (Appendix 2), that would also qualify as threatened nationally, but are not yet included on the national threatened species list. An additional 54 species are listed under the category of 'rare' within the relevant jurisdiction (Appendix 3), that would also have this status nationally, but are not on the national threatened species list. There are seven species of orchids included on the national EPBC Act threatened species lists that are not included in the relevant state/territory threatened species list (Appendix 4). An additional two threatened orchids on the national list, *Dendrobium brachypus* (Endl.) H.G. Reichb. and *Phreatia paleata* H.G. Reichb., occur on Australia's island territories not under state/territory jurisdiction.

TABLE 2. Number of extinct, threatened and rare orchid species in Australia by jurisdiction.

	NAT	NT	Qld	NSW	ACT	Vic	Tas	SA	WA
EX	7	0	3	0			2	0	0
EW	0								
CR	23			0			2		
EN	91	2	19	33	3		47	45	
VU	74	6	25	21			3	31	
RA			60				15	28	36
TH						75			
CD	0								
Totals	**195**	**8**	**107**	**54**	**3**	**75**	**69**	**104**	**36**

Shaded boxes indicate where the conservation status category is not used in the national/state/territory legislation

Abbreviations

Rows are conservation status categories: EX = extinct; EW = extinct in the Wild; CR = critically endangered; EN = endangered; VU = vulnerable; RA = rare; TH = threatened; CD = conservation cependent

Columns are jurisdictions: NAT = National; NT = Northern Territory; Qld = Queensland; NSW = New South Wales; ACT = Australian Capital Territory; Vic = Victoria; Tas = Tasmania; SA = South Australia; WA = Western Australia.

REGIONAL ASSESSMENTS – For South Australia, the assessment by Bates (2006) indicated 146 orchids of 'Conservation Concern', compared with 104 listed under state legislation (Table 3). For Victoria, the two assessments indicated a total of 240 (Backhouse & Cameron 2005) and 257 orchids (DSE 2005) of

TABLE 3. Assessments of the numbers of orchids of 'conservation concern' in South Australia.

Category	NPWA	Bates (2006)
extinct	0	14
endangered	45	51
vulnerable	31	20
threatened	76	71
rare	28	48
data deficient	0	13
Total conservation concern	104	146

NPWA = National Parks & Wildlife Act 1972, South Australia.

'Conservation Concern', compared with just 75 orchids listed under state legislation (Table 4).

Discussion

The national EPBC Act includes 195 orchid species listed as extinct or threatened nationally (Table 2), which is about 12% of the Australian orchid flora. The state and territory regional threatened species lists include another 52 species that are listed as extinct or threatened within their jurisdiction, that would also qualify as threatened nationally, but are not yet included on the national threatened species list. If these species are added, the total national extinct/threatened orchid count is 15% of the nation's orchids. An additional 54 species are listed under the category of 'rare' within the relevant jurisdiction that would have this status at the national level. Therefore, there is a total of 301 species of orchids of conservation concern (= extinct, threatened or rare) at the national level, that are currently listed on national and regional biodiversity conservation legislation. This is 18% of the Australian orchid flora. Based solely on a comparison of the official national and state/territory legislative threatened species lists, it appears that the official national list underestimates the real number of threatened orchids by at least 50 species, and perhaps as many as 100 or more species.

The comparisons of the comprehensive reviews of the conservation status of the orchid flora of South Australia (Bates 2006) and Victoria (Backhouse & Cameron 2005, DSE 2005) with listed threatened orchids in those states provides further evidence that official lists are a substantial underestimate of the actual number of threatened orchids. In South Australia, the number listed under the NPW Act may be an underestimate of the actual number of extinct, threatened or rare orchids by at least 42 species, based on Bates (2006) (Table 3). There is good evidence to suggest that at least 14 (and perhaps as many as 20) orchid species have become extinct in that state (Bates 2006), yet no orchid is currently listed as extinct under the NPW Act. In Victoria, there appears to be a much larger discrepancy between the official and actual number of threatened orchids. There are at least 150 extinct or threatened orchid species (DSE 2005), and perhaps over 200 (Backhouse & Cameron 2005), compared with only 75 species listed as threatened under the Victorian Flora and Fauna Guarantee Act 1988.

TABLE 4. Assessments of the numbers of orchids of 'conservation concern' in Victoria.

Category	FFGA	B&C 2005	DSE 2005
extinct		11	11
critically endangered		79	
edangered		26	95
vlnerable		92	49
threatened	**75**	**197**	**144**
near threatened		8	
data deficient		24	
insufficiently known			43
rare			59
Total conservation concern	**75**	**240**	**257**

Shaded boxes indicate where the conservation status category is not used in the assessment system
FFGA = Flora and Fauna Guarantee Act 1988, Victoria.
B&C 2005 = Backhouse & Cameron 2005.

These figures also do not take account of recent taxonomic advances. For example, 90 new species of orchids for Australia were described in late 2006 (Jones 2006b, 2006c, Jones & Clements 2006, Jones & Rouse 2006, Jones *et. al* 2006), of which at least one was considered extinct and 53 considered threatened. Most of these species have yet to be included on any threatened species list.

This review and assessments strongly suggests that official lists of threatened orchids at both the national and state/territory level are a substantial underesti-

mate of the actual numbers of threatened orchids in Australia. There are several possible reasons for this large discrepancy.

LISTING PROCESS – The process to officially list a species as threatened can take a considerable period of time. Several jurisdictions have a similar listing process that includes the following steps:

- a species is nominated for listing;
- the nomination is assessed by a scientific reference committee;
- the committee makes a recommendation to list (or not list) to the relevant government minister;
- the recommendation is advertised for public comment;
- the committee makes a final recommendation to list (or not list);
- the government makes the listing.

In Victoria, under the FFG Act, the process takes a minimum of nine months in straightforward cases, and can take well over a year in complicated or contentious cases. Therefore, the listing process can lag well behind an initial assessment of threat. It is likely to be some years yet before the national threatened species list includes most or all orchids currently considered threatened.

TAXONOMY – There are many known but undescribed orchid species considered threatened (eg. Backhouse & Cameron 2005, DSE 2005, Bates 2006) that are not yet listed. If a species is not formally named, there is an understandable reluctance to list an essentially unknown entity. However, several jurisdictions have provision for listing known but undescribed species, and some undescribed orchids are listed at the state and national level (see Appendix 1). The recent description of over 50 new species considered threatened nationally (Jones 2006b, 2006c, Jones & Clements 2006, Jones & Rouse 2006, Jones *et. al* 2006) will greatly assist the prospects for these species being eventually listed.

DIFFERING THREAT ASSESSMENTS – Differing assessments of conservation status under different jurisdictions, and the use of different assessment systems, may also be hindering the listing of nationally threatened orchids. While there may be some commonality between terminology used in most lists (eg. extinct, endangered, vulnerable), definitions do vary. Several state/territory lists still use 'rare', which is not regarded as a category of threat, and at least 54 species are rare at the national level. At least some of these species listed may well qualify for listing as threatened under national legislation. For instance, an assessment of the 45 orchid species considered rare in Victoria (DSE 2005) against IUCN 2001 categories found that 32 (71%) of these species were threatened, with 30 being assessed as vulnerable (Backhouse & Cameron 2005). The Queensland Government is phasing out the term 'rare' from its legislative list and current rare species will be reassessed to determine if any are threatened, although this won't happen until 2010. Even some publications use inconsistent standards when describing conservation status. For instance, Riley and Banks (2002) often use 'rare' in combination with a threat category (eg. rare and endangered; rare and vulnerable) when describing conservation status. Jones (2006b) uses IUCN 2001 for some conservation status assessments of threatened orchids, and a system known as AROTs (Australian Rare or Threatened Species, *sensu* Briggs & Leigh 1996) for other assessments, with several potentially threatened orchids being assessed as rare.

Recommendations

This review and assessment of national and state/territory lists of threatened orchids in Australia has highlighted several deficiencies in the multiplicity of systems adopted by the different jurisdictions. Following are five recommendations proposed to improve the system for listing threatened orchids in Australia:

1. *Undertake a comprehensive national review of the conservation status of Australia's orchids.*

A comprehensive national review and assessment of the conservation status of Australian orchids is highly desirable, as the most suitable and rapid way to bring national and regional threatened species lists up to date. This review should be undertaken using a single assessment system, preferably the IUCN Red List Categories and Criteria (IUCN 2001). This national review could easily be adapted for state/territory jurisdictions through the use of the IUCN regional assessment guidelines (IUCN 2003) in undertaking

the conservation status assessments. The national conservation status assessment could be modelled in the form of similar assessments in the national 'Action Plan' series undertaken for Australia's vertebrate fauna (eg. Wager & Jackson 1993, Bannister *et al.* 1996, Garnett & Crowley 2000) and butterflies (Sands & New 2003). This review can form the basis of a concerted approach to formally listing the large number of threatened orchids not currently on official legislative lists.

2. *Adopt a common set of categories and criteria for describing the conservation status of Australian orchids at both the national and regional (state/territory) level.*

Currently the state, territory and national governments use different systems for describing conservation status, which can make for vague, confusing or conflicting definitions, and comparisons between lists difficult. Even in some recent publications, conservation status assessments can be confusing. The IUCN Red List Categories and Criteria (IUCN 2001) are the current international standard, and have been adapted for use in the national EPBC Act. This is the most logical system to use for assessing conservation status, especially with the guidelines for application at the regional level (IUCN 2003). This would provide a common language at the regional and national level in communicating conservation status of threatened orchids.

3. *Streamline administrative processes to facilitate cross listings of threatened orchids.*

Threatened orchids listed under state/territory legislation currently have to go through a separate process for listing under national legislation. There are at least 50 orchid species listed under state/territory legislation that would qualify for listing under national legislation, and at least another 50 species listed as rare that would possibly qualify for national listing. At the current rate of listing, it will take several years for these species to be assessed and listed at the national level. It is highly desirable that, in situations where a threatened species is listed in a state or territory, and is endemic to that state/territory, there is a simple administrative process to quickly list these species under national legislation.

4. *Streamline administrative processes to accommodate changes in taxonomy.*

There have been many taxonomic changes affecting Australian orchids, especially changes to genus names, in recent years, and further changes are likely. At least 50 threatened orchid species are currently listed under an invalid scientific name, with some of these names having changed several years ago. Current systems for listing threatened species under biodiversity conservation legislation at both the state/territory and national level do not adequately deal with taxonomic changes. For threatened species that have had a name change since listing, this effectively requires a nomination to delist under the old name and then nomination for relisting under the new name. A process is clearly required to rapidly update the legislative threatened species lists to accommodate advances in science and taxonomy. A mechanism for linking listed species names with official taxonomic checklists maintained by state/territory and national herbaria would provide an efficient way for dealing with taxonomic changes.

5. *Prepare national and regional (state/territory) advisory lists of threatened orchids.*

The preparation of non-legislative 'advisory' lists of threatened orchids is a useful way of fairly quickly accommodating changes in taxonomy, information and conservation status. These are peer-reviewed, and can be updated much more rapidly than is the case with legislative lists. For instance, the Victorian threatened species advisory lists (DSE 2003, 2005) are revised every 2–3 years. While these advisory lists have no formal legislative standing, they are very useful as guides to the categories and number of threatened species, and can be used to highlight those species requiring formal listing under legislation.

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LITERATURE CITED

Backhouse, G. and Cameron, D. 2005. Application of IUCN Red List categories in determining the conserva-

tion status of the native orchids in Victoria, Australia. Selbyana 26(1,2) : 58–74.

Bannister, J.L., Kemper, C.M. and Warneke, R.M. 1996. The action plan for Australian cetaceans. Environment Australia, Canberra.

Bates, R. 2006 (editor). South Australian native orchids. Native Orchid Society of South Australia Inc. Electronic version.

Briggs, J.D and Leigh, J.H. 1996. Rare or threatened Australian Plants, revised edition. CSIRO and Australian Nature Conservation Agency, Canberra.

DSE 2003. Advisory list of threatened vertebrate fauna in Victoria – 2003. Department of Sustainability and Environment, Victoria.

DSE 2005. Advisory list of rare or threatened plants in Victoria – 2005. Department of Sustainability and Environment, Victoria.

Garnett, S. T. and Crowley, G.M. 2000. The action plan for Australian birds. Environment Australia, Canberra.

IUCN 2001. IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Gland and Cambridge.

IUCN 2003. Guidleines of Application of IUCN Red List Criteria at Regional Levels. Version 3.0. IUCN Species Survival Commission. IUCN, Gland and Cambridge.

Jones, D.L. 2006a. Native orchids of Australia. Reed New Holland, Frenchs Forest.

Jones, D.L. 2006b. Miscellaneous new species of Australian Orchidaceae. *Aust. Orchid Res.* 5 : 45–111.

Jones, D.L. 2006c. Towards a revision of *Bunochilus* D.L.Jones & M.A.Clem. *Aust. Orchid Res.* 5 : 112–142.

Jones, D.L. and Clements, M.A. 2006. Fourteen new taxa of Orchidaceae from northern and eastern Australia and two new combinations from New Guinea. *Aust. Orchid Res.* 5 : 2–33.

Jones, D.L. and Rouse, D.T. 2006. Fourteen new species of *Prasophyllum* from eastern Australia. *Aust. Orchid Res.* 5 : 143–168.

Jones, D.L., Clements, M.A. and Sharma, Ish. 2006. Towards a revision of the *Thelychiton speciosus* group. *Aust. Orchid Res.* 5 : 34–44.

Riley, J. and Banks, D.P. 2002. Orchids of Australia. University of New South Wales Press, Sydney.

Sands, D.P.A. and New, T.R. 2002. The action plan for Australian butterflies. Environment Australia, Canberra.

Wager, R. and Jackson, P. 1993. The action plan for Australian freshwater fishes. Environment Australia, Canberra.

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APPENDIX 1. Australian orchids listed as extinct, threatened or rare under national and state/territory biodiversity conservation legislation.

N	Species	NAT	NT	Qld	NSW	ACT	Vic	Tas	SA	WA
1	*Acianthus amplexicaulis*			RA						
2	*Acianthus collinus*						TH			
3	*Acianthus ledwardii*	EX		EX						
4	*Acianthus sublestis*			RA						
5	*Acriopsis javanica*			VU						
6	*Aphyllorchis anomala*			RA						
7	*Aphyllorchis queenslandica*			RA						
8	*Bulbophyllum argyropus*			RA						
9	*Bulbophyllum blumei*			EN						
10	*Bulbophyllum boonjee*			RA						
11	*Bulbophyllum globuliforme*	VU		RA	VU					
12	*Bulbophyllum gracillimum*	VU		VU						
13	*Bulbophyllum grandimesense*			RA						
14	*Bulbophyllum longiflorum*	VU		VU						
15	*Bulbophyllum weinthalii*			VU						
16	*Bulbophyllum windsorense*			RA						
17	*Bulbophyllum wolfei*			RA						
18	*Cadetia collinsii*			RA						
19	*Cadetia wariana*			RA						
20	*Caladenia actensis*	CR				EN				
21	*Caladenia amoena*	EN					TH			
22	*Caladenia anthracina*	CR						CR		
23	*Caladenia arenaria*	EN			EN					
24	*Caladenia argocalla*	EN							EN	
25	*Caladenia atroclavia*	EN		EN						
26	*Caladenia audasii*	EN					TH		EN	
27	*Caladenia aurantiaca*							EN		
28	*Caladenia australis*							EN		
29	*Caladenia barbarella*	VU								RA
30	*Caladenia behrii*	EN							EN	
31	*Caladenia bicalliata*								RA	
32	*Caladenia brachyscapa*	EX					TH	EN		
33	*Caladenia brumalis*	VU							VU	
34	*Caladenia bryceana* subsp. *bryceana*	EN								RA
35	*Caladenia bryceana* subsp. *cracens*	VU								RA
36	*Caladenia busselliana*	EN								RA
37	*Caladenia caesarea* subsp. *maritima*	EN								RA
38	*Caladenia calcicola*	VU					TH		VU	
39	*Caladenia campbellii*	CR						CR		
40	*Caladenia cardiochila*							EX		
41	*Caladenia caudata*	VU						RA		
42	*Caladenia christineae*	VU								RA
43	*Caladenia clavigera*								EN	
44	*Caladenia cleistogama*								VU	
45	*Caladenia colorata*	EN							EN	
46	*Caladenia concolor*	VU			EN		TH			
47	*Caladenia conferta*								EN	
48	*Caladenia congesta*							EN	RA	
49	*Caladenia cruciformis*						TH			
50	*Caladenia cucullata*								RA	
51	*Caladenia dienema*	CR						VU		**WA**

N	Species	NAT	NT	Qld	NSW	ACT	Vic	Tas	SA	WA
52	*Caladenia dorrienii*	EN								RA
53	*Caladenia drakeoides*	EN								RA
54	*Caladenia elegans*	EN								RA
55	*Caladenia excelsa*	EN								RA
56	*Caladenia filamentosa*							RA		
57	*Caladenia formosa*	VU					TH		VU	
58	*Caladenia fragrantissima*						TH		RA	
59	*Caladenia fulva*	EN					TH			
60	*Caladenia gladiolata*	EN							EN	
61	*Caladenia gracilis*								EN	
62	*Caladenia graniticola*									RA
63	*Caladenia harringtoniae*	VU								RA
64	*Caladenia hastata*	EN					TH			
65	*Caladenia hoffmanii*	EN								RA
66	*Caladenia huegelii*	EN								RA
67	*Caladenia insularis*	VU					TH			
68	*Caladenia lindleyana*	CR						EN		
69	*Caladenia lowanensis*	EN					TH			
70	*Caladenia macroclavia*	EN							EN	
71	*Caladenia magnifica*						TH			
72	*Caladenia melanema*									RA
73	*Caladenia minor*								RA	
74	*Caladenia necrophylla*								RA	
75	*Caladenia ornata*	VU								
76	*Caladenia orientalis*	EN					TH			
77	*Caladenia ovata*	VU							VU	
78	*Caladenia pallida*	CR						EN		
79	*Caladenia parva*								EN	
80	*Caladenia patersonii*							VU		
81	*Caladenia pilotensis*						TH			
82	*Caladenia porphyrea*				EN					
83	*Caladenia procera*									RA
84	*Caladenia prolata*							EN		
85	*Caladenia pumila*	EX					TH			
86	*Caladenia pusilla*							RA		
87	*Caladenia richardsiorum*	EN							EN	
88	*Caladenia rigida*	EN							EN	
89	*Caladenia robinsonii*	EN					TH			
90	*Caladenia rosella*	EN					TH			
91	*Caladenia saggicola*	CR						EN		
92	*Caladenia subulata*	EN					TH			
93	*Caladenia sylvicola*	CR						EN		
94	*Caladenia tensa*	EN								
95	*Caladenia tessellate*	VU			EN					
96	*Caladenia thysanochila*	EN					TH			
97	*Caladenia tonellii*	CR						EN		
98	*Caladenia toxochila*						TH			
99	*Caladenia valida*						TH		RA	
100	*Caladenia venusta*								VU	
101	*Caladenia versicolor*	VU					TH		VU	
102	*Caladenia viridescens*	EN								RA
103	*Caladenia vulgaris*								RA	
104	*Caladenia wanosa*	VU								RA
105	*Caladenia williamsiae*									RA

N	Species	NAT	NT	Qld	NSW	ACT	Vic	Tas	SA	WA
106	*Caladenia winfieldii*	EN								RA
107	*Caladenia woolcockiorum*	VU							VU	
108	*Caladenia xanthochila*	EN					TH		EN	
109	*Caladenia xantholeuca*	EN							EN	
110	*Caladenia* species 'Bordertown'								EN	
111	*Caladenia colorata*						TH			
112	*Caladenia* species 'Finniss'								EN	
113	*Caladenia* species aff. *fragrantissima*						TH			
114	*Caladenia* species 'Jarrah forest'	VU								
115	*Caladenia* species 'Koolunga'								EN	
116	*Caladenia* species aff. *rosella*						TH			
117	*Caladenia* species aff. *venusta*	CR					TH			
118	*Caleana major*								VU	
119	*Calochilus caeruleus*		VU							
120	*Calochilus campestris*								RA	
121	*Calochilus cupreus*								EN	
122	*Calochilus paludosus*								VU	
123	*Calochilus psednus*	EN		EN						
124	*Calochilus richiae*	EN					TH			
125	*Chiloglottis anaticeps*				EN					
126	*Chiloglottis cornuta*								EN	
127	*Chiloglottis longiclavata*			RA						
128	*Chiloglottis platyptera*				VU					
129	*Chiloglottis seminuda*						TH			
130	*Chiloglottis sphyrnoides*			VU						
131	*Chiloglottis trapeziformis*								EN	
132	*Corunastylis brachystachya*	EN						EN		
133	*Corunastylis ectopa*	CR				EN				
134	*Corunastylis firthii*	CR						EN		
135	*Corunastylis morrisii*							EN	EN	
136	*Corunastylis nuda*							RA		
137	*Corunastylis nudiscapa*							EX		
138	*Corybas abellianus*			RA						
139	*Corybas dentatus*	VU							EN	
140	*Corybas despectans*						TH			
141	*Corybas fordhamii*							EN	EN	
142	*Corybas montanus*	VU		VU						
143	*Corybas neocaledonicus*			RA						
144	*Corybas unguiculatus*								RA	
145	*Corybas* species aff. *diemenicus* (coastal)						TH			
146	*Corybas* species 'Finniss'	EN							EN	
147	*Cryptostylis erecta*						TH			
148	*Cryptostylis hunteriana*	VU			VU		TH			
149	*Cryptostylis leptochila*							EN		
150	*Cryptostylis subulata*								VU	
151	*Cyrtostylis robusta*							RA		
152	*Dendrobium antennatum*	EN		EN						
153	*Dendrobium bigibbum*	VU		VU						
154	*Dendrobium brachypus*	EN								
155	*Dendrobium callitrophilum*	VU		VU						
156	*Dendrobium carronii*	VU		VU						
157	*Dendrobium fellowsii*			RA						
158	*Dendrobium johannis*	VU		VU						
159	*Dendrobium lithocola*	EN		EN						

N	Species	NAT	NT	Qld	NSW	ACT	Vic	Tas	SA	WA
160	*Dendrobium malbrownii*			RA						
161	*Dendrobium melaleucaphilum*				EN					
162	*Dendrobium mirbelianum*	EN		EN						
163	*Dendrobium nindii*	EN		EN						
164	*Dendrobium phalaenopsis*	VU		VU						
165	*Dendrobium schneiderae* var. *schneiderae*			RA						
166	*Dendrobium speciosum*						TH			
167	*Dendrobium superbiens*	VU		VU						
168	*Didymoplexis pallens*			RA						
169	*Diplocaulobium masonii*	EX		EX						
170	*Dipodium campanulatum*								VU	
171	*Dipodium hamiltonianum*						TH			
172	*Dipodium pardalinum*								VU	
173	*Dipodium pictum*	EN		EN						
174	*Dipodium pulchellum*			RA						
175	*Diuris aequalis*	VU			EN					
176	*Diuris arenaria*				EN					
177	*Diuris behrii*								RA	
178	*Diuris bracteata*	EX			EN					
179	*Diuris brevifolia*								RA	
180	*Diuris chryseopsis*								EN	
181	*Diuris dendrobioides*						TH			
182	*Diuris disposita*				EN					
183	*Diuris drummondii*	VU								RA
184	*Diuris flavescens*				EN					
185	*Diuris fragrantissima*	EN					TH			
186	*Diuris lanceolata*	EN						EN		
187	*Diuris micrantha*	VU								RA
188	*Diuris ochroma*	VU			EN		TH			
189	*Diuris oporina*			RA						
190	*Diuris palustris*						TH	EN		
191	*Diuris parvipetala*			RA						
192	*Diuris pedunculata*	EN			EN					
193	*Diuris praecox*	VU			VU					
194	*Diuris punctata* var. *punctata*						TH		EN	
195	*Diuris purdiei*	EN								RA
196	*Diuris sheaffiana*	VU								
197	*Diuris sulphurea*								RA	
198	*Diuris tricolor*				VU		TH			
199	*Diuris venosa*	VU			VU					
200	*Diuris* species aff. *lanceolata*	EN					TH			
201	*Diuris* species aff. *chrysantha* 'Byron				EN					
202	Bay'				EN					
203	*Diuris* species 'Oaklands'			RA						
204	*Dockrillia wassellii*	VU								
205	*Drakaea concolor*	EN								RA
206	*Drakaea confluens*	EN								RA
207	*Drakaea elastica*	EN								RA
208	*Drakaea isolata*	VU								RA
209	*Drakaea micrantha*	EN								RA
210	*Epiblema grandiflorum* var. *cyaneum*			RA						RA
211	*Eria dischorensis*			RA						
212	*Eria irukandjiana*			RA						
213	*Eulophia bicallosa*			RA						

N	Species	NAT	NT	Qld	NSW	ACT	Vic	Tas	SA	WA
214	*Eulophia zollingeri*			RA						
215	*Gastrodia crebrifolia*			RA						
216	*Gastrodia queenslandica*			RA						
217	*Gastrodia urceolata*								RA	
218	*Gastrodia sesamoides*								VU	
219	*Gastrodia vescula*			RA						
220	*Genoplesium alticolum*				VU					
221	*Genoplesium baueri*								EN	
222	*Genoplesium ciliatum*								VU	
223	*Genoplesium despectans*				EN					
224	*Genoplesium insignis*			RA						
225	*Genoplesium pedersonii*	EN			EN					
226	*Genoplesium plumosum*	EN			EN					
227	*Genoplesium rhyoliticum*			RA						
228	*Genoplesium sigmoideum*				EN					
229	*Genoplesium superbum*	VU			VU					
230	*Genoplesium vernale*	EN		EN						
231	*Genoplesium tectum*			RA						
232	*Genoplesium validum*				EN					
233	*Geodorum densiflorum*	VU		VU						
234	*Grastidium tozerense*			RA						
235	*Habenaria divaricata*			EN						
236	*Habenaria harroldii*			RA						
237	*Habenaria hymenophylla*	EN		EN						
238	*Habenaria macraithii*		EN							
239	*Habenaria rumphii*			RA						
240	*Habenaria xanthantha*							RA		
241	*Hydrorchis orbicularis*			RA						
242	*Liparis condylobulbon*			RA						
243	*Liparis simmondsii*		VU							
244	*Luisia teretifolia*		VU							
245	*Malaxis latifolia*		VU							
246	*Malaxis marsupichila*							RA	RA	
247	*Microtidium atratum*	EN			EN					
248	*Microtis angusii*									
249	*Microtis globula*								RA	RA
250	*Microtis orbicularis*								RA	
251	*Microtis rara*			RA						
252	*Nervilia crociformis*		EN							
253	*Nervilia plicata*	EX		EX						
254	*Oberonia attenuata*			RA						
255	*Oberonia carnosa*				EN					
256	*Oberonia complanata*				VU					
257	*Oberonia titania*			RA						
258	*Pachystoma pubescens*			RA						
259	*Papillilabium beckleri*						TH		EN	
260	*Paracaleana* sp. aff. *nigrita*	EN								
261	*Paracaleana dixonii*								VU	RA
262	*Paracaleana minor*			RA	VU					
263	*Peristeranthus hillii*			RA						
264	*Peristylus banfieldii*	EN		EN	EN					
265	*Phaius australis*	EN		EN						
266	*Phaius bernaysii*	VU		VU						
267	*Phaius pictus*	EN		EN	EN					

N	Species	NAT	NT	Qld	NSW	ACT	Vic	Tas	SA	WA
268	*Phaius tancarvilleae*	EN		EN						
269	*Phalaenopsis rosenstromii*	EN								
270	*Phreatia paleata*	VU		VU						
271	*Pomatocalpa marsupiale*	EN			EN					
272	*Prasophyllum affine*	EN						EN		
273	*Prasophyllum amoenum*	EN						EN		
274	*Prasophyllum apoxychilum*								RA	
275	*Prasophyllum australe*				EN					
276	*Prasophyllum bagoensis*								VU	
277	*Prasophyllum calcicola*			RA						
278	*Prasophyllum campestre*	CR						EN		
279	*Prasophyllum castaneum*	EN					TH			
280	*Prasophyllum chasmogamum*	VU								
281	*Prasophyllum colemaniae*								RA	
282	*Prasophyllum constrictum*	EN					TH			
283	*Prasophyllum diversiflorum*			RA						
284	*Prasophyllum exilis*	CR						EN		
285	*Prasophyllum favonium*								RA	
286	*Prasophyllum fecundum*						TH			
287	*Prasophyllum fitzgeraldii*						TH			
288	*Prasophyllum fosteri*	EN					TH		EN	
289	*Prasophyllum frenchii*	VU			VU					
290	*Prasophyllum fuscum*								RA	
291	*Prasophyllum goldsackii*							EN		
292	*Prasophyllum incorrectum*			RA						
293	*Prasophyllum incompositum*						TH			
294	*Prasophyllum litorale*	CR						EN		
295	*Prasophyllum milfordense*							EN		
296	*Prasophyllum montanum*	VU					TH			
297	*Prasophyllum morganii*						TH			
298	*Prasophyllum niphopedium*								RA	
299	*Prasophyllum occultans*	CR						EN		
300	*Prasophyllum olidum*	VU							VU	
301	*Prasophyllum pallidum*	CR						EN		
302	*Prasophyllum perangustum*	EN			EN					
303	*Prasophyllum petilum*								VU	
304	*Prasophyllum pruinosum*	CR				EN		EN		
305	*Prasophyllum pulchellum*							EN		
306	*Prasophyllum pyriforme*				VU					
307	*Prasophyllum retroflexum*	CR						EN		
308	*Prasophyllum robustum*	EN						VU		
309	*Prasophyllum secutum*	VU							EN	
310	*Prasophyllum spicatum*	CR						EN		
311	*Prasophyllum stellatum*	EN					TH			
312	*Prasophyllum suaveolens*	EN					TH			
313	*Prasophyllum subbisectu*						TH			
314	*Prasophyllum suttonii*							RA		
315	*Prasophyllum tadgellianum*							EN		
316	*Prasophyllum taphanyx*	EN						EN		
317	*Prasophyllum tunbridgense*	EN			EN					
318	*Prasophyllum uroglossum*	VU							VU	
319	*Prasophyllum validum*	VU		VU						
320	*Prasophyllum wallum*				EN					
321	*Prasophyllum* species 'Majors Creek'						TH			

N	*Prasophyllum* species 'Nagambie'	NAT	NT	Qld	NSW	ACT	Vic	Tas	SA	WA
322	*Pterostylis aenigma*	EN					TH			
323	*Pterostylis arenicola*	VU							VU	
324	*Pterostylis atriola*	EN						EN		
325	*Pterostylis baptistii*						TH			
326	*Pterostylis basaltica*	EN					TH			
327	*Pterostylis bicornis*	VU		VU						
328	*Pterostylis bryophila*								EN	
329	*Pterostylis chaetophora*			EN						
330	*Pterostylis cheraphila*	VU					TH			
331	*Pterostylis chlorogramma*	VU								
332	*Pterostylis cobarensis*	VU			VU					
333	*Pterostylis commutata*	CR						EN		
334	*Pterostylis concinna*								EN	
335	*Pterostylis cucullata*	VU			VU		TH	EN	VU	
336	*Pterostylis curta*								RA	
337	*Pterostylis cycnocephala*							EN		
338	*Pterostylis despectans*	EN					TH			
339	*Pterostylis elegans*				VU					
340	*Pterostylis falcata*							RA		
341	*Pterostylis foliata*								RA	
342	*Pterostylis furcata*								EN	
343	*Pterostylis gibbosa*	EN			EN					
344	*Pterostylis grandiflora*							RA		
345	*Pterostylis longicurva*			RA						
346	*Pterostylis metcalfei*				EN					
347	*Pterostylis nigricans*			RA	VU					
348	*Pterostylis pratensis*	VU								
349	*Pterostylis pulchella*	VU			VU					
350	*Pterostylis rubenachii*	EN						EN		
351	*Pterostylis sanguinea*							RA		
352	*Pterostylis saxicola*	EN			EN					
353	*Pterostylis setifera*			RA					EN	
354	*Pterostylis squamata*							RA		
355	*Pterostylis tasmanica*								VU	
356	*Pterostylis tenuissima*	VU							VU	
357	*Pterostylis truncata*						TH			
358	*Pterostylis tunstallii*							EN		
359	*Pterostylis uliginosa*								EN	
360	*Pterostylis valida*	EX					TH			
361	*Pterostylis wapstrarum*	CR						EN		
362	*Pterostylis woollsii*			RA			TH			
363	*Pterostylis xerophila*	VU					TH		VU	
364	*Pterostylis ziegeleri*	EN						EN		
365	*Pterostylis* species 'Gundiah'			RA						
366	*Pterostylis* species aff. *boormanii*						TH			
367	*Pterostylis* species 'Botany Bay'	EN			EN					
368	*Pterostylis* species 'Broughton Gorge'								EN	
369	*Pterostylis* species 'Eyre Peninsula'	VU							VU	
370	*Pterostylis* species 'Halbury'	EN							EN	
371	*Pterostylis* species 'Hale'	EN							EN	
372	*Pterostylis* species 'Mt Bryan'								EN	
373	*Pterostylis* species 'Mt Olinthus'								EN	
374	*Pterostylis* species 'Mt Victoria Uranium Mine'								VU	
375	*Pterostylis* species 'Northampton'	EN								RA

N	Species	NAT	NT	Qld	NSW	ACT	Vic	Tas	SA	WA
376	*Pterostylis* species 'Oratan Rock'								VU	
377	*Pterostylis* species aff. *parviflora*								VU	
378	*Pterostylis* species 'Sandy Creek'								VU	
379	*Rhinerrhiza moorei*	VU		VU						
380	*Rhizanthella slateri*			RA	VU					
381	*Robiquetia wasselii*			RA						
382	*Sarcochilus dilatatus*				EN					
383	*Sarcochilus falcatus*						TH			
384	*Sarcochilus fitzgeraldii*	VU		EN	VU					
385	*Sarcochilus hartmannii*	VU		VU	VU					
386	*Sarcochilus hirticalcar*	VU		VU						
387	*Sarcochilus roseus*	VU		VU						
388	*Sarcochilus weinthalii*	VU		EN	VU					
389	*Schoenorchis sarcophylla*			RA						
390	*Spathoglottis paulinea*			RA						
391	*Spathoglottis plicata*	VU		VU						
392	*Spiranthes australis*								RA	
393	*Taeniophyllum confertum*			RA						
394	*Taeniophyllum lobatum*			RA						
395	*Taeniophyllum muelleri*	VU								
396	*Thelasis carinata*			RA						
397	*Thelymitra antennifera*							EN		
398	*Thelymitra benthamiana*							EN		
399	*Thelymitra bracteata*							EN		
400	*Thelymitra carnea*								RA	
401	*Thelymitra circumsepta*								EN	
402	*Thelymitra deadmaniarum*	EN								RA
403	*Thelymitra epipactoides*	EN					TH		EN	
404	*Thelymitra flexuosa*								RA	
405	*Thelymitra gregaria*						TH			
406	*Thelymitra hiemalis*						TH			
407	*Thelymitra holmesii*							RA	VU	
408	*Thelymitra jonesii*							EN		
409	*Thelymitra mackibbinii*	VU					TH			
410	*Thelymitra malvina*							EN	EN	
411	*Thelymitra matthewsii*	VU					TH		EN	
412	*Thelymitra merraniae*						TH			
413	*Thelymitra mucida*							RA	RA	
414	*Thelymitra psammophila*	VU								RA
415	*Thelymitra stellata*	EN								RA
416	*Thelymitra venosa*								EN	
417	*Thrixspermum congestum*		VU							
418	*Thynninorchis huntiana*							EN		
419	*Thynninorchis nothofagicola*	CR						EN		
420	*Trichoglottis australiensis*	VU		VU						
421	*Vanda hindsii*	VU		VU						
422	*Vrydagzynea paludosa*	EN		EN						
423	*Zeuxine oblonga*		VU							
424	*Zeuxine polygonoides*	VU		VU						
	Totals	**195**	**8**	**107**	**54**	**3**	**75**	**69**	**104**	

NAT = National; NT = Northern Territory; Qld = Queensland; NSW = New South Wales; ACT = Australian Capital Territory; Vic = Victoria; Tas = Tasmania; SA = South Australia; WA = Western Australia.

APPENDIX 2. Nationally threatened orchids listed at the state/territory level but not at the national level.

N	Species	Qld	NSW	Vic	Tas	SA
1	*Acriopsis javanica*	VU				
2	*Caladenia conferta*					EN
3	*Caladenia cruciformis*			TH		
4	*Caladenia fragrantissima*			TH		RA
5	*Caladenia magnifica*			TH		
6	*Caladenia patersonii*				VU	
7	*Caladenia pilotensis*			TH		
8	*Caladenia porphyrea*		EN			
9	*Caladenia valida*			TH		RA
10	*Caladenia* species 'Bordertown'					EN
11	*Caladenia* species 'Finniss'					EN
12	*Caladenia* species aff. *fragrantissima*			TH		
13	*Caladenia* species 'Koolunga'					EN
14	*Caladenia* species aff. *rosella*			TH		
15	*Chiloglottis anaticeps*		EN			
16	*Chiloglottis platyptera*		VU			
17	*Genoplesium insignis*		EN			
18	*Genoplesium superbum*		EN			
19	*Diuris arenaria*		EN			
20	*Diuris* species aff. *chrysantha* 'Byron Bay'		EN			
21	*Diuris* species 'Oaklands'		EN			
22	*Gastrodia vescula*					VU
23	*Genoplesium baueri*		VU			
24	*Habenaria harroldii*	EN				
25	*Peristeranthus hillii*	RA	VU			
26	*Prasophyllum bagoensis*		EN			
27	*Prasophyllum fosteri*			TH		
28	*Prasophyllum incorrectum*				EN	
29	*Prasophyllum litorale*			TH		
30	*Prasophyllum niphopedium*			TH		
31	*Prasophyllum pruinosum*					VU
32	*Prasophyllum retroflexum*		VU			
33	*Prasophyllum suttonii*			TH		
34	*Prasophyllum taphanyx*				EN	
35	*Prasophyllum* species 'Majors Creek'		EN			
36	*Prasophyllum* species 'Nagambie'			TH		
37	*Pterostylis bryophila*					EN
38	*Pterostylis elegans*		VU			
39	*Pterostylis metcalfei*		EN			
40	*Pterostylis nigricans*	RA	VU			
41	*Pterostylis* species 'Broughton Gorge'					EN
42	*Pterostylis* species 'Mt Bryan'					EN
43	*Pterostylis* species 'Mt Olinthus'					EN
44	*Pterostylis* species 'Mt Victoria Uranium Mine'					VU
45	*Pterostylis* species 'Oratan Rock'					VU
46	*Pterostylis* species aff. *parviflora*					VU
47	*Pterostylis* species 'Sandy Creek'					VU
48	*Rhizanthella slateri*	RA	VU			
49	*Thelymitra gregaria*			TH		
50	*Thelymitra hiemalis*			TH		
51	*Thelymitra jonesii*				EN	
52	*Thelymitra merraniae*			TH		

Qld = Queensland; NSW = New South Wales; Vic = Victoria; Tas = Tasmania; SA = South Australia.

APPENDIX 3. Nationally rare orchids listed at the state/territory level but not at the national level.

N	Species	Qld	SA	WA
1	*Acianthus amplexicaulis*	RA		
2	*Acianthus sublestis*	RA		
3	*Aphyllorchis anomala*	RA		
4	*Aphyllorchis queenslandica*	RA		
5	*Bulbophyllum blumei*	EN		
6	*Bulbophyllum boonjee*	RA		
7	*Bulbophyllum grandimesense*	RA		
8	*Bulbophyllum windsorense*	RA		
9	*Bulbophyllum wolfei*	RA		
10	*Cadetia collinsii*	RA		
11	*Cadetia wariana*	RA		
12	*Caladenia graniticola*			RA
13	*Caladenia melanema*			RA
14	*Caladenia minor*		RA	
15	*Caladenia necrophylla*		RA	
16	*Caladenia procera*			RA
17	*Chiloglottis longiclavata*	RA		
18	*Corybas abellianus*	RA		
19	*Corybas neocaledonicus*	RA		
20	*Dendrobium fellowsii*	RA		
21	*Dendrobium malbrownii*	RA		
22	*Diuris oporina*	RA		
23	*Diuris parvipetala*	RA		
24	*Dockrillia wassellii*	RA		
25	*Eria dischorensis*	RA		
26	*Eria irukandjiana*	RA		
27	*Eulophia zollingeri*	RA		
28	*Gastrodia crebrifolia*	RA		
29	*Gastrodia queenslandica*	RA		
30	*Gastrodia urceolata*	RA		
31	*Genoplesium alticolum*	RA		
32	*Genoplesium pedersonii*	RA		
33	*Genoplesium sigmoideum*	RA		
34	*Genoplesium validum*	RA		
35	*Habenaria divaricata*	RA		
36	*Habenaria xanthantha*	RA		
37	*Liparis condylobulbon*	RA		
38	*Liparis simmondsii*	RA		
39	*Microtis globula*			RA
40	*Nervilia crociformis*	RA		
41	*Oberonia carnosa*	RA		
42	*Peristylus banfieldii*	RA		
43	*Prasophyllum constrictum*		RA	
44	*Prasophyllum fecundum*		RA	
45	*Prasophyllum goldsackii*		RA	
46	*Prasophyllum incompositum*	RA		
47	*Prasophyllum occultans*		RA	
48	*Pterostylis species* 'Gundiah'	RA		
49	*Robiquetia wasselii*	RA		
50	*Schoenorchis sarcophylla*	RA		
51	*Spathoglottis paulinea*	RA		
52	*Taeniophyllum confertum*	RA		
53	*Taeniophyllum lobatum*	RA		
54	*Thelasis carinata*	RA		

Qld = Queensland; SA = South Australia; WA = Western Australia

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UNDERSTANDING THE DISTRIBUTION OF THREE SPECIES OF EPIPHYTIC ORCHIDS IN TEMPERATE AUSTRALIAN RAINFOREST BY INVESTIGATION OF THEIR HOST AND FUNGAL ASSOCIATES

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KEY WORDS: epiphyte, Aeridinae, orchid mycorrhizal fungi, *Ceratobasidium*, chemotropism

Introduction

Understanding the environmental constraints that affect species distributions are critical to the maintenance of biodiversity. The abundance of epiphytic organisms, those that grow on another substrate, such as a tree or rock, is a direct consequence of the availability and distribution of these substrates (Ackerman *et al.* 1989). In the case of epiphytic orchids it is also due to the presence of orchid mycorrhizal fungi (OMF). For an orchid, crucial to its germination and establishment, is its association with an OMF. The OMF provides a carbon source to the developing orchid embryo (Rasmussen 1995). Although reciprocal carbon transfer has been demonstrated in mature plants of a green, terrestrial, orchid species, *Goodyera repens* (Cameron *et al.* 2006), it is generally believed that OMF receive no immediate benefit from their association with orchids. Therefore, it would appear intuitive that orchids would associate with all OMF available within their local environment and that they would actively seek this association.

In this investigation we sought to ascertain the nature of the relationship between three closely related, co-occurring species of epiphytic, Aeridinae (= Sarcanthinae) orchids, their OMF, and their phorophytes (host trees). The orchid study species: *Sarcochilus hillii*, *Sarcochilus parviflorus* and *Plectorrhiza tridentata* are all small, monopodial epiphytes found on trees and shrubs in temperate rainforest gullies. The null hypothesis that we are testing is that these three orchid species are randomly distributed throughout their forest habitat.

More specifically we are addressing the following questions:

- Do these three epiphytic orchid species exhibit a random distribution across the woody plants of the forest?
- Do these three orchid species associate with all OMF within their local environment?
- Do the OMF of these orchid species differ in their ability to stimulate germination amongst these species?
- Are these OMF actively attracted towards the seed of these three orchid species?

Methods

To address these questions we surveyed four sites where these three orchid species co-occur in temperate south-eastern Australia. The woody plant composition of the forests and the associations of these three orchid species with their phorophytes were determined using a maximum likelihood model. Generalised Linear Mixed Models (GLMMs) were used to detect preferences for physical features of the phorophyte and local environment of these orchid species.

To ensure adequate sampling of the OMF of each orchid species, ten members of each species were surveyed from two sites. To investigate the diversity of OMF on the preferred phorophyte, five orchids that were sampled of each species were on the most common host. Earlier research indicated that other members of these orchid genera associated with the Ceratobasidiaceae within the form-genus *Rhizoctonia* (Warcup 1981). We also targeted *Rhizoctonia*-like fungi when we isolated OMF from the roots of these orchids. Verification that the isolated fungi were capable of stimulating orchid germination (and therefore, were indeed OMF) was determined by germination trials. Genetic identification of the fungal associates was conducted by sequencing the nuclear ribosomal internal transcribed spacer (Gardes & Bruns 1993) and the mitochondrial large subunit (White *et al.* 1990), and through the amplification of dispersed repetitive DNA sequences (Versalovic *et al.* 1991). Finally, to determine if the fungi were actively attracted towards orchid seed, chemotropism trials were conducted and the amount of fungal growth towards test and control aliquots (of seed and water respectively) was compared using Paired t-tests.

Results and discussion

Backhousia myrtifolia was the most common tree at most sites and was the dominant phorophyte species for all three orchid species, significantly so for *S. parviflorus* and *P. tridentata*. All three orchid species preferred a phorophyte with moderate to high moss cover. Despite these similarities, distinct differences in the distribution patterns were detected for each species of orchid.

These three orchid species differed in the composition of their phorophyte flora: *S. hillii's* distribution approximated a random distribution which reflected that of the rainforests' tree species composition; *P. tridentata* exhibited a strong bias towards *B. myrtifolia*, although was otherwise on the broadest range of phorophyte species; and *S. parviflorus* had the narrowest range of phorophytes, exhibiting clear preferences for and against particular woody plant species. However, the 'species' of phorophyte was not the only correlate with orchid presence, each orchid species exhibited non-random patterns in their proximity to moss and location on their phorophytes. Characteristics of the phorophyte that had the greatest effect on the size and reproductive potential of the orchids, as measured by the size and number of leaves and number of inflorescences, were independent of the species of the phorophyte.

We expected that these distributional differences would reflect distinct OMF associations with each orchid species; however, whilst different OMF were found in association with these orchids it has not explained the difference in phorophyte species association. All OMF isolated from these three orchid species belonged to two distinct clades, groups, within the genus *Ceratobasidium,* recognised as clade L and clade K. All three orchid species associated with clade K, but only *S. hillii* was found with clade L. This did not, however, explain the random distribution of *S. hillii*, as members of both fungal clades were isolated from orchids on the common phorophyte, *B. myrtifolia*. Additionally, germination trials revealed that even though both groups of fungi were not naturally found in association with *S. parviflorus* and *P. tridentata*, members of each OMF clade could stimulate germination in all three orchid species *ex situ*.

Furthermore, the chemotropism experiment revealed that members of both OMF clades were attracted towards viable orchid seed. This is the first experiment, that we know of, that has demonstrated that orchid mycorrhizal fungi is actively attracted to orchid seed.

Conclusion

Each orchid species clearly demonstrated characteristic preferences for phorophyte species or features, indicative of specific ecological niches. They did not exhibit a random distribution throughout the forest. Furthermore, despite exposure to multiple potential OMF, *S. parviflorus* and *P. tridentata* were only found in association with a restricted subset of those available in their local environment. This is despite *ex situ* results indicating that there is no inherent physiological reason why they do not associate with both groups of OMF, and the fact that both clades of fungi are actively attracted to orchid seed.

These results typify the intrigue around this family

of plants. For example, why would *S. parviflorus* and *P. tridentata* attract, but not utilise all OMF within their ecosystem? Possible explanations and ideas for further study will be discussed.

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LITERATURE CITED

Ackerman, J.D., A.M. Montalvo & A.M. Vera. 1989. Epiphyte host specificity of *Encyclia krugii*, a Puerto Rican endemic orchid. Lindleyana 4**:** 74-77.

Cameron, D.D., J.R. Leake & D.J. Read. 2006. Mutualistic mycorrhiza in orchids: evidence from plant-fungus carbon and nitrogen transfers in the green-leaved terrestrial orchid *Goodyera repens*. New Phytol. 171**:** 405-416.

Gardes, M. & T.D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol. 2**:** 113-118.

Rasmussen, H.N. 1995. Terrestrial Orchids from seed to mycotrophic plant. Melbourne, Australia, Cambridge University Press.

Versalovic, J., T. Koeuth & J.R. Lupski. 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucl. Acids Res. 19**:** 6823-6831.

Warcup, J.H. 1981. The mycorrhizal relationships of Australian orchids. New Phytol. 87**:** 371-381.

White, T.J., T. Bruns, S. Lee & J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White (eds.), PCR Protocols: A guide to methods and applications. San Diego, Academic Press.

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RARE PLANT RESTORATION ON LONG PINE KEY

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The Long Pine Key area of Everglades National Park (Florida) is critical habitat for a large number of rare plant species including two candidates for federal listing and several dozen listed as endangered or threatened by the state of Florida. In addition, there are nineteen species present in the Long Pine Key area that are critically imperiled in South Florida and six species historically documented from the area that may be extinct in the continental United States (Gann et al., 2002).

Most of the critically imperiled species have been poorly studied, their distributions in Everglades National Park are not adequately documented, and their growth requirements are little known. Historically, water flow through Long Pine Key was concentrated in a series of short hydro-periods that traversed prairies the area in a north-south direction. Artificial drainage is believed to have affected Long Pine Key habitats by increasing the frequency and intensity of fires which damage hammocks, and by increasing exposures to freezing temperatures through the lowering of water levels and the opening up of hammock canopies. Marie Selby Botanical Gardens is assisting with the reintroduction and augmentation of epiphytes and lithophytic ferns.

Presently MSBG is propagating three ferns and two orchids: *Pecluma plumula* (Humb. & Bonpl. ex Willd.) M.G. Price, the plumed rockcap fern, *Adiantum melanoleucum* Willd., the fragrant maidenhair fern, *Thelypteris reticulata* (L.) Proctor, the lattice-vein fern, two orchids *Brassia caudata* Lindl., the Spider orchid, and *Oncidium ensatum* Lindl., Florida dancing-lady orchid. Augmentation trials will be initiated, using measures of plant community habitat and environmental variables to help identify favorable reintroduction sites.

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THE STATUS OF ORCHID CONSERVATION IN CHINA

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KEY WORDS: ecosystem types, endemic group, conservation, natural reserves, orchid flora, biological characters.

Orchids are a flag group in plant conservation. China has not a rich orchid flora, with only about 1200 specie in about 170 genera, but it is distinguished by having a wider range of broad ecosystem types. On orchid vegetative morphology, a feature reflecting environmental conditions, China has equal numbers of terrestrial and epiphytic (including lithophytic) genera. This feature is quite different from the tropical zone where epiphytic orchids are majority and from the temperate regions where terrestrial orchids predominate, and is unique in the world orchids flora. Of the Chinese orchid flora, there are 502 species in 98 genera being endemic to China, and 26 genera in which have more than half of the total species being endemic to China. Moreover, there are some world famous ornamental or medicinal orchids in China, such as *Paphiopedilum*, *Cypripedium*, *Cymbidium*, *Pleione*, *Holcoglossum* and *Dendrobium*. And the Chinese *Cymbidium*s are among the best of the favorable ornamental orchids in China. Some *Cymbidium* plantations, as well as much more private yards, have been set up in China mainland, Taiwan and Hong Kong. Many species of *Cymbidium*, thus, have become seriously endangered or quite rare or even extinct in some areas.

Recently years, Chinese Government has paid great attention to orchid conservation. General policies have been carried out and some efforts have been made to improve the situation. A long term project launched by Chinese government has carried out to protect the wildlife, orchids are one of the key species in the project. on *in situ* conservation, the natural reserves of varies kinds have be increased to 2349, covering 150 million hectares of area, more than 15 per cent of the total territory. Moreover, those natural reserves will cover almost the upper reaches of China's major rivers and areas featuring intact bio-diversity and the richest orchid flora. Particularly, one special orchids nature reserve was set up in Guangxi Province in 2005. About *ex situ* conservation, the State Forestry Administration of China has set up one *ex situ* conservation center in Shengzheng, Guangdong Province. Also as one important part of the China's southwest wild biological germplasm resource bank, the orchids seed bank project has been started in 2004. Morover, a reintroduction project of *Doritis pulcherrima* Lindl. was carrying out in Hainan Island. However, the conservation of the orchids is in fact a complicated problem, not only depending on education and economic development, but also to a large extent on the biological characters of the orchids themselves. It needs a comprehensive study of ecology, population biology, pollination biology, breeding biology and other biological branches.

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EPIPHYTE ORCHID DIVERSITY IN A YUNGAS MONTANE FOREST IN THE COTAPATA NATIONAL PARK AND INTEGRATED MANAGEMENT NATURAL AREA, LA PAZ – BOLIVIA

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KEY WORDS: epiphyte orchids, diversity, Yungas montane forest, Cotapata, Bolivia

Introduction

In Bolivia the works focused on the study of the epiphyte vegetation are few and recent. This lack of knowledge is being filled by investigations like those of Ibisch (1996) about the flora and epiphyte vegetation; Acebey & Krömer (2001), Acebey *et al.* (2003), Altamirano & Fernandez (2003) and Miranda (2005), who worked in the diversity and ecology of vascular and not vascular epiphytes.

In Bolivia a total of 20.000 species of angiosperms has calculated (Beck 1998), Vásquez *et al.* (2003) estimates between 2000 to 3000 of these plants are orchids, actually there is a list with approximately 1500 species, of which near 1200 have been identified (Vásquez *et al.* 2003). Sixty percent of the species and 80% of the endemic orchids are concentrated in the region of the Yungas that does not occupy more than 4% of the surface of the country (Vásquez *et al*. 2003).

The area of the Yungas in La Paz is one of the most explored places of Bolivia (Beck 1993). A continuous work has contributed to a great, but non total knowledge of the flora, for example, a study of epiphytes in the montane forests of the Cotapata National Park and Integrated Management Natural Area (PN-ANMI) has a total of 292 species in an inventory of three parcels of 0,32 ha. each one, of which the orchids with a 44% represent the most important family (Krömer & Gradstein 2003).

Since May of 2005 is developed the project: "Study of the potential of sustainable use of epiphytes in the PN-ANMI Cotapata", with the initial objective of know more on the diversity of epiphytes orchids in the montane forests of Yungas of this protected area. This work presents the preliminary results of the inventories developed in this project.

Study area

The Cotapata PN-ANMI (fig. 1) is located in the provinces Murillo and Nor Yungas of the department of La Paz, with a surface of 40.000 ha and goes from the 1100 to 5600 m of altitude (Ribera 1995). This wide altitudinal gradient originates a great variety of climates and types of vegetation in an area of heterogenous topography; also affected from old times by human activities. The principal forest formations are the cloudy forest (2400-3400 m) characterized by a cool and very humid climate and the humid forest of Yungas (2400-1200 m) which has a conspicuous dry time (Ribera 1995). Bach *et al*. (2003) report 3000 mm and 10,1 ºC for the cloudy forest and 2550 mm and 13-17,2 ºC for the humid forest of Yungas. In the south sector of the protected area starts two pre-Columbian paths; these are constructed across the core of both forests:

- Chojllapata, which mostly crosses the crest of the mountains (3400-1300 m) until arriving at the locality of El Chairo;
- Sillutinkara, which crosses in its beginning the valley of the Coscapa river (3400-2000 m) and meet the the path of El Choro, in the proximities of Sandillani.

Bajo Hornuni (1800 m), located at the bottom of the Hornuni hill, in front of Sandillani, this is covered by a humid montane forest of Yungas.

Methods

The field work was conducted from July 2005 to May 2006, in zones of non- disturbed forest. For the inventory of epiphyte orchids of understory and canopy, 3 to 5 non-permanent plots of 20 x 20 m was installed each 100 altitudinal meters (modified of Krömer

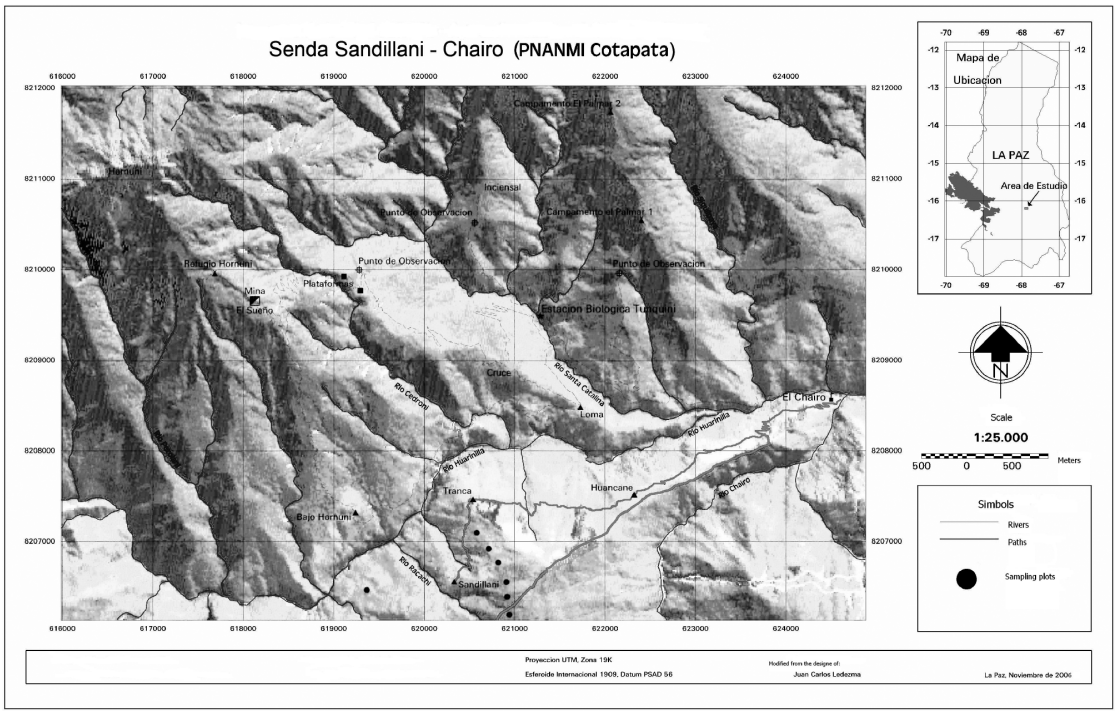

FIGURE 1. Ubication map of the sampling zones inside Cotapata Nacional Park.

2003) and a representative tree for each altitudinal range of 100 m, inside or near to a plot, which was evaluated using the techniques described by Perry (1978). Fertile and sterile orchids were collected and used for the analysis. Sterile individuals or with fruits were marked with marking tapes and respective code of collection. These plants were transplanted to a single trunk (called: storing zones) inside or close the surveyed plot, with the purpose of maintaining alive collections and obtaining fertile material that helps its identification. To complete the floristic inventory, general collections were made throughout the pre-Columbian paths. In addition orchid flowers were collected and preserved in small bottles with a solution of 70% of alcohol. All the samples are deposited in the Herbario Nacional of Bolivia (LPB).

Results and discussion

From the evaluation of 47 non permanent plots, 13 phorophyts and general collections we registered 255 species of epiphyte orchids. The most representative genera are *Stelis* Sw. (19%), *Pleurothallis* R.Br. (15%), *Epidendrum* L. (14%), and *Maxillaria* Ruiz & Pav. (13%) (Fig. 2). Acebey & Krömer (2001) in foodmontane forests of Bolivia and Nowicki (2001), in a cloudy forest of Ecuador, found similar proportions. On the other hand Vasquez e*t al.* (2004) indicates that in Bolivia the genera *Pleurothallis* and *Epidendrum* constitutes the most diverse taxa.

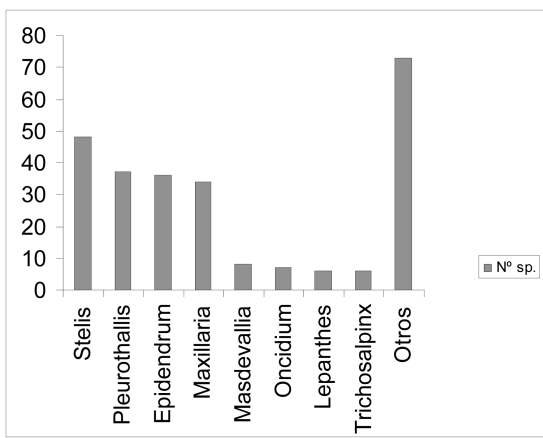

FIGURE 2. Diversity of the most important genera in the study zone.

From our results is clear to point out that in the genera *Epidendrum* and *Maxillaria* there are a great proportion of unidentified species; the same happens in the preliminary list of orchids of Bolivia (Vasquez *et al.* 2003), this unidentified orchids could represent new species or new registries for Bolivia.

In addition, new records at local and regional level stand out, for example *Odontoglossum vierlingii* Senghas, considered endemic to the department of Cochabamba, was found in the study area. Similarly, *Prostechea pulchra* Dodson & W.E. Higgins, found in dispersed populations, until now has been only recorded in the humid montane forests of Ecuador and Peru (Higgins & Dodson 2001); now we found it in our study zone, in a moderately disturbated montane forest, on the edge of the Silluntinkara path, at 2100 m approximately. In the genus: *Epidendrum* L., *Maxillaria* Ruiz & Pav., *Cyrtochilum* Kunth, *Stelis* Sw. and *Masdevallia* Ruiz & Pav. we found many unidentified specimens, therefore is highly probable that exist new species (Vásquez R., pers. comm. 2006). With more sampling we hope to find new registries for the zone and new species for science.

Our results show a great diversity in a relatively wide gradient. For example Krömer *et al.* (2005) in a altitudinal range of 350 to 4000 m above sea level, registered 314 species of orchids. Also compared with the study of Krömer (2003) for the region, we registered the double of species but in a wide altitudinal range. These highlight the importance of the zone for the diversity of orchids. The high diversity of the study zone could be explained for the interaction between heterogeneous topography and the wide altitudinal gradient, both generating a variety of climates and different habitats able for support diverse vegetation. Still more, the deforestation originated for the continuous use of this forest from pre-Columbian time to recent times has a negative impact on the diversity of orchids (Krömer 2003); although this large diversity is an indicator for the high resilience of the forest.

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LITERATURE CITED

Acebey, A. & T. Krömer. 2001. Diversidad y distribución vertical de epífitas en los alrededores del campamento río Eslabón y de la laguna Chalalán, Parque Nacional Madidi, Depto. La Paz, Bolivia. Rev. Soc. Boliv. Bot. 3: 104-123.

Acebey, A., S.R Gradstein & T. Krömer. 2003. Species richness and habitat diversification of bryophytes in submontane rain forest and fallows of Bolivia. J. Trop. Ecol. 19: 9-18.

Altamirano, S. & E. Fernández. 2003. Diversidad y distribución vertical de epífitas en bosques amazónicos de tierra firme del TIPNIS (Territorio Indígena y Parque Nacional Isiboro Sécure) Cochabamba, Bolivia. Rev. Bol. Ecol. 14: 67-80.

Bach, K., M. Schawe, S. Beck, G. Gerold, S.R. Gradstein & M. Moraes. 2003. Vegetación, suelos y clima en los diferentes pisos altitudinales de un bosque montano de Yungas, Bolivia: Primeros resultados. Ecología en Bolivia 38(1): 3-14.

Beck, S. G., T. J. Killeen & E. García. 1993. Vegetación de Bolivia. Pp. 6-23 *in*: T.J. Killeen, E. García & S.G. Beck (eds.). Guía de árboles de Bolivia. La Paz, Herbario Nacional de Bolivia-Missouri Botanical Garden, Quipus S.R.L., La Paz.

Beck, S.G. 1998. Floristic inventory of Bolivia - An indispensable contribution to sustainable development? Pp. 243-268 *in*: W. Barthlott & M. Winiger (eds.). Biodiversity – A challenge for development research and policy. Springer – Verlag, Berlin.

Higgins, W.E. & C.H.Dodson. 2001. *Prostechea pulchra*: A New Name for an Andean Orchid. Selbyana 22(2): 128-130.

Ibisch, P. 1996. Neotropische epiphytendiversität-das Beispiel Bolivien, Martina Galunder-Verlag, Wiehl, 357 p.

Krömer, T. 2003. Diversität und Ökologie der vaskulären Epiphyten in primären und sekundären Bergwäldern Boliviens. Cuvillier Verlarg, Göttingen.

Krömer, T. & R. Gradstein. 2003. Species richness of vascular epiphytes in two primary forests and fallows in the Bolivian Andes. Selbyana 24(2): 195–195.

Krömer T., M. Kessler, S.R. Gradstein & A Acebey. 2005.

Local-scale diversity patterns of vascular epiphytes along an elevational gradient in the Andes. Journal of Biogeography 32(10): 1799-1809.

Miranda, F. 2005. Diversidad alfa, beta y distribución vertical de epífitas vasculares en dos rangos altitudinales de un bosque yungueño pluvial submontano en el ANMI Apolobamba, La Paz – Bolivia. Tesis de grado, Carrera de Biología, UMSA, La Paz, 55 p.

Nowicki, C. 2001. Epífitas vasculares de la Reserva Otonga. Pp. 115-155 *in*: J. Nieder& W. Barthlott (eds.). Canopo plants and animals of the Otonga Reserve (Ecuador). Results of a joint research project Bonn-Quito, funded by the Volkswagen Foundation.

Perry, D.R. 1978. A method of access into the crowns of emergent and canopy trees. Biotropica 10 (2) : 155-157.

Ribera, M. 1995. Aspectos ecológicos, del uso de la tierra y conservación en el Parque Nacional y Área Natural de Manejo Integrado Cotapata. Pp. 1-84 *in*: C. Morales (ed.). Caminos de Cotapata. Instituto de Ecología / FUND-ECO / FONAMA-EIA, La Paz.

Vasquez, R., P.L. Ibisch & B. Gerkmann. 2003. Diversity of Bolivian Orchidaceae – a challenge for taxonomic, floristic and conservation research. Organisms, Diversity and Evolution 3 (2) : 93-102. Electr. Suppl.: Appendix 1 (preliminary list of Bolivian orchid species, http://www.senckenberg.uni-frankfurt.de/odes/03-4.pdf).

Vásquez, R., P.L. Ibisch, A. Ley & C. Nowicki. 2004. Los géneros y especies de las Laeliinae. Pp: 80-335 *in*: R. Vásquez & P.L. Ibisch (eds.). Orquídeas de Bolivia. Diversidad y estado de conservación. Vol II Laeliinae, Polystachyinae, Sobraliinae con actualización y complementación de Pleurothallidinae. Editorial FAN, Santa Cruz-Bolivia.

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GEOLOGICAL PROCESSES AND ORCHID BIOGEOGRAPHY WITH APPLICATIONS TO SOUTHEAST CENTRAL AMERICA

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KEY WORDS: geological processes, orchid biogeography, speciation, volcanic activity, subduction, tectonic plates

Introduction

This contribution owes its origins to a paper and presentation by Dr. Calloway H. Dodson at the Second International Conference on Neotropical Orchidology held in San José, Costa Rica in May of 2003 (Dodson 2003). Dr. Dodson outlined some of the reasons to suspect that regional geological factors may play important roles in orchid speciation and biogeography and gave examples from the northwestern South America. He also suggested that evolutionary change in orchid might occur over fairly short time periods, perhaps even as short as decades, centuries or millennia (Dodson 2003, SHK lecture notes).

These ideas stimulated the author, a professional earth scientist, to begin thinking about how these exciting ideas could begin to be tested in Costa Rica neighboring and Central American countries, an area that has drawn him to return frequently over the last decade. The present contribution is a proposal for integrating geological observations, such as the chronology of arc volcanic activity in Nicaragua, Costa Rica, and Panama, in hypothesis forming and testing of the geographic distribution of orchids (and possibly other biota). I initially focus on comparisons between orchid inventories on the windward slopes of mountainous regions (elevation > 1000 m) with high rainfall (> 1-2 m) in tropical regions, the so-called tropical cloud forests. These regions represent the *tropical pre-montane rain forest* to *lower montane tropical rain forest* life zones of Holdridge (1967) and the *montane* vegetation zone applied to Costa Rica and Panama by Dressler (1993). An important message of this paper is that such tropical mountainous regions are not necessarily static, but may change in elevation over geologic time due to active tectonic deformation and uplift and that the presence of active volcanism in a mountain range may also introduce additional chemical factors, such as volcanic gases, acid rain, and volcanic soils, and also physical factors, such as interruption of gene flow by explosive eruptions and coverage by their air fall products such as ash (tephra), lava flows, and lahars (volcanic mudflows). Thus over a given geological time interval, forests may be slowly increasing in elevation by tectonic uplift or by the accumulation of volcanic products such as steep-sided stratovolcanoes (built from both lavas and tephra), or by down-slope accumulations of lava flows or lahars. Mountains may also lose elevation by erosion or by tectonic subsidence. As we shall see, tropical Central America shows an extraordinarily high level of tectonic and volcanic history that has changed its geography and, by implication, climate, life zones, and likely orchid distribution. My working hypothesis put forward for testing is that orchid adaptations to these changes may have led to the development of new species and endemism in this region.

Geological Background

The region of southeast Central America (Nicaragua, Costa Rica and Panama) and NW Colombia is a center of profound geological changes during the late Cenozoic (Pliocene to present, 0-5 million years ago) (see excellent summaries in Denyer and Kussmaul 2000, Denyer *et al.* 2003). It is one of the most active tectonic regions of the world, being at the nexus of four major moving tectonic plates, the Cocos, Nazca, Caribbean, and South America, and three smaller microplates: the Coiba, Panama, and North Andean. As such, it abounds in geologically young mountain belts from Nicaragua to

the northern Andes, active volcanic chains, and earthquakes and earthquake belts related to the motions of these plates.

Subduction of the Cocos plate under Central America is marked by the Middle America Trench off the Pacific coast that results from the down bending of the Cocos plate that subsequently descends at various angles under Central America from Mexico to western Panama. This descent produces an inclined zone of earthquakes that represent earthquake slip between the sinking Cocos Plate and the plates above (the North American and Caribbean plates) as well as internal seismic deformation in the Cocos plate. Subduction has also built a nearly continuous chain of active arc volcanoes from Mexico to SE Costa Rica that is thought to represent the effects of water released from the Cocos plate as it heats up during descent into hot mantle and induces melting in the hot mantle above the sinking plate (often termed a "slab").

In addition to the first-order deformation pattern associated with subduction, SE Central America displays clear evidence for internal deformation in the plates above the Cocos slab (Caribbean and Panama), deformation that builds tectonic mountains and has affected the history of seaways that segmented Central America in the recent geologic past. Finally, the Cocos plate is decorated by volcanic islands, seamounts, and the Cocos Volcanic Ridge that have been produced by the Galapagos Volcanic Hot Spot that also built the Galopagos Islands. The hot-spot islands, ridges, and plateaus built on the Cocos plate have been moving toward the Middle America Trench with time. These have collided or are colliding with the Central American Isthmus in Costa Rica and western Panama and some of these structures have accreted to the Isthmus (e.g., the Nicoya and Osa Peninsulas). Thus the geographic positions and elevation ranges of mountain belts have rapidly changed in this region over the last 5-10 million years and these changes undoubtedly have led to important changes in rainfall distributions and temperature and in the continuity of life zones. I now discuss the following potential implications of these regional plate-tectonic processes for the biogeography of SE Central America.

Geological Events Possibly Relevant to Orchid Science in the Region

Chief among important geological events that have accompanied these processes are:

1. The well known early Pliocene closing of the Panama Seaway and the subsequent rise of the Panamanian Cordillera from the seafloor and their effects on ocean circulation, weather, and faunal exchange across the Isthmus.
2. Less well known is the Holocene (since 10,000 years ago) opening and partial closing of the Nicaraguan Seaway, represented presently by the lowland from the Gulf of Fonseca, to Lakes Managua and Nicaragua and the San Juan River Valley. This lowland is thought by Costa Rican consulting geologist Roberto Protti (personal communication 2007) to have represented subsidence in a graben (a valley created by a fault-bounded down-dropped block) that was flooded by the sea.
3. The geologically recent migration toward the Middle American Trench of offshore volcanic islands (e.g., Cocos Island), flat-topped seamounts (former islands, such as the Fisher Seamount), and the Cocos volcanic ridge associated with volcanic processes at the Galapagos Hot Spot. Island speciation from continental forebears like that which has occurred the Galapagos Islands could possibly lead to reverse gene flow to continents as plate motion brings oceanic island crust close to the Middle America Trench. Subsequent collision of such terrains with Costa Rica (Caribbean plate) probably produced stresses and deformation in Costa Rica that raised tectonic mountain belts, such as the Talamanca cordillera.
4. The late Cenozoic rise of the Central Volcanic Range (CVR) in Costa Rica, one of the youngest arc volcanic mountain ranges in the world (largely in the Pleistocene to the present, 1.64 million years to the present) and hence its geologically recent effects on topography, rainfall distribution, air quality (from volcanic gases), and soil chemistries.
5. The late Tertiary (about 5 million years ago) cessation of volcanism in older, presently non-volcanic cordillera, such as the Talamanca, SW of the CVR and the Tilaran Cordillera, SE of the CVR.
6. The ongoing uplift of the Coast Ranges south of

the Talamanca and the non-volcanic Matama cordillera east of the CVR.

The above changes not only are potentially important in orchid gene flow, but also may influence through volcanic chemistry such processes as orchid mutagenesis, pollination, and germination. As such, their understanding may lead to useful hypotheses concerning orchid biogeography and to purposeful orchid surveys to test them.

The majority of the pristine forests of Costa Rica and Nicaragua have disappeared largely through deforestation. Conservation of the remaining orchid ecosystems is a critical requirement in order for such investigations of the origins of orchid biogeography to be successful. Orchid surveys directed to this particular end therefore should be purposeful. The ongoing multi-year survey of orchids at the Bosque de Paz Biological Reserve began in June of 2004 (See Kirby 2003, Muñoz and Kirby this volume). It is believed to be the first attempt at conducting a comprehensive survey of orchid species in the active Central Volcanic Range in Costa Rica above 1500 m elevation. I compare the identified species in the genera from this Survey that I feel are likely nearly complete after 2.6 years of monthly collection, description, and identification with those from Carpentera, Tapanti, San Ramón, and Monte Verde, all pre-montane to montane rain forest environments in Costa Rica.

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LITERATURE CITED

Denyer, P. & S. Kussmaul (eds). 2000. Geología de Costa Rica. ISBN 9977-66-118-9. Editorial Technologica de Costa Rica. 515 p.

Denyer, P., W. Montero & G.E. Alvarado. 2003. Atlas Tectónico de Costa Rica. Universidad de Costa Rica, San José, CR. Serie Reportes técnicos. 81 p.

Dodson, C.H. 2003. Why are there so many orchid species?. I[er] Congreso International de Orquideología Neotropical, 20-25 Mayo, 2003, Proceedings Issue in Lankesteriana 7: 99-103.

Dressler, R.L. 1993. Field Guide to the Orchids of Costa Rica and Panama. Cornell University. New York. 374 p.

Holdridge, L. 1967. Life Zone Ecology. Tropical Science Center, Costa Rica. 89 p.

Kirby, S.H. 2003. Neotropical orchid ecotourism: Educational experience of an orchid neophyte at the Bosque de Paz Biological Reserve, Central Volcanic Range, Costa Rica, I[er] Congreso International de Orquideología Neotropical, 20-25 Mayo, 2003, Proceedings Issue *in* Lankesteriana 7: 121-124.

Muñoz, M. & S. H. Kirby. 2007. An orchid inventory and conservation project at Bosque de Paz Biological Reserve, Upper Rio Toro Valley, Alajuela, Costa Rica, Proceedings of the 3[rd] International Orchid Conservation Conference *in* Lankesteriana (this issue)

Ossenbach, C., F. Pupulin & R.L. Dressler. 2007. Orchids of the Central American Isthmus: Checklist and conservation status. Unpublished.

Protti, R. 2007. Posible interrupción parcial del paso terrestre en Centroamérica durante la transgresión Flandriense (Holoceno medio). Personal communication.

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DIVERSIDAD DE ORQUIDEAS EN EL "PARQUE NACIONAL IZTACCIHUATL-POPOCATÉPETL" (MÉXICO) Y SUS ÁREAS DE INFLUENCIA

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ABSTRACT. Iztaccíhuatl and Popocatépetl National Park is 25,679 ha in size and it comprises the Transmexican Neo-volcanic strip. The floristic wealth of the Park approximately represents 4% of the flora of the country and the Orchidaceae is one of the families of this flora. Mexico has 1400 orchid species including in 159 genera; the importance at a world-wide level of this flora increases when around 900 species exist only in Mexico. In 1996, 25 orchid species were reported for the Park without a field register. These species and their populations can drastically have varied and in some cases disappeared due to the alterations of the habitat and to the extraction of the plants of the last decades. In June of 2001 we started the present project which the main objective has been to obtain by means of work in field, the updated listing of the orchid flora of this Park at different seasons of the year, as well as to determine the diversity of species, plant development stage, their altitudinal distribution and abundance. After 5 years of work 39 species have been located, 25 species are new registers to the Park and there were confirm only 14 species of the 1996 listing.

KEY WORDS: diversidad, orquideoflora, Parque Nacional, lista de orquídeas, México, hábito de crecimiento, Iztaccíhuatl, Popocatépetl

Introducción

En México la riqueza orquideológica se manifiesta con más de 1200 especies (Hágsater *et al.* 2005), el porcentaje de endemismos es alto, aproximadamente 35% de especies y 8% de géneros (Soto 1988) y generalmente se delimita a cadenas montañosas o a zonas de extensión reducida. Soto *et al.* (2001) mencionan que no existe información precisa del número que integran dentro del Sistema Nacional de Áreas Naturales Protegidas (CONANP 2004), más sin embargo, éste puede ser del 80%. Dentro de la gran diversidad de la flora que se puede encontrar dentro del Parque Nacional Iztaccíhuatl-Popocatépetl (PNIP) y su zona de influencia, están los integrantes de la familia Orchidaceae, que son un recurso natural, un patrimonio de nuestro país y deben utilizarse racionalmente para su mantenimiento y conservación (Soto & Hagsater 1990). Chávez y Trigo (1996) reportaron para el Parque 14 géneros y 24 especies de orquídeas, de las cuales seis son endémicas de México, cuatro son de distribución exclusiva del centro, sur de México y dos del Eje Neovolcánico Transversal. Se puede afirmar que aún no se han registrado el total de las especies para la zona, probablemente la presencia de las especies y de sus poblaciones pudieron haber disminuido drásticamente y en algunos casos haber desaparecido a causa de alteraciones o destrucción de sus hábitats en las últimas décadas, más que por la sobrecolecta de las plantas, como generalmente se cree (Soto & Hagsater 1990). Debido a la falta de un inventario actualizado y completo sobre la diversidad orquideológica del Parque, se plantearon como objetivos del presente trabajo actualizar el listado de especies, determinar estacional y altitudinalmente las especies localizadas, así como su distribución y abundancia.

Metodología

UBICACION GEOGRAFICA. El área de estudio se ubica en los límites de tres entidades federativas del país: México, Morelos y Puebla, dentro de la zona templado-subhúmeda del país, que comprende los principales sis-

temas montañosos de México como el Eje Neovolcánico Transmexicano, región donde se encuentran los volcanes Iztaccíhuatl y Popocatépetl. Se sitúa entre las coordenadas geográficas 18º59' y 19º16'25" de latitud N y 98º34'54" y 98º16'25" de longitud W, cuenta con una superficie de 25,679 ha (Vargas 1997). Las comunidades vegetales relevantes que predominan son el Bosque de Pino, Pino-Encino, Oyamel, Páramo de altura y Zacatonal. Por su ubicación y por el marcado gradiente altitudinal que presenta, posee una gran diversidad de hábitats lo que refleja su riqueza florística que representa aproximadamente el 4% de la flora del país (25,000 taxas de plantas vasculares).

SITIOS DE PROSPECCION Y REGISTRO. En junio del 2001 al 2006 se realizaron salidas mensuales a campo para efectuar recorridos aleatorios en diversos sitios del PNIP y sus áreas de influencia en un rango altitudinal desde los 1500 m hasta los 4000 m sobre el nivel del mar. Se utilizaron cartas topográficas escala 1:50 000 del Instituto Nacional de Estadística Geográfica e Informática (INEGI 1998), correspondientes a los municipios de los tres estados para ubicar los sitios de prospección de orquídeas. En cada zona donde se localizaron orquídeas se registró la ubicación georeferenciada, altitud y tipo de vegetación. Se determinó el hábito de crecimiento, distribución y estado fenológico de las orquídeas. En algunos casos la identificación de las especies se llevó a cabo por el personal del Herbario de la Asociación Mexicana de Orquideología (AMO).

Resultados

COMPOSICION Y DIVERSIDAD. Se localizaron y determinaron 39 especies de orquídeas en el área de estudio, incluidas en 20 géneros, el número de especies varió en cada uno y fue mayor para *Malaxis* Sol. ex Sw., *Bletia* Ruiz & Pav., *Corallorhiza* Gagnebin y *Schiedeella* Schltr. (Tabla 1). Se localizaron 10 géneros y 14 especies de las orquídeas reportados por Chávez y Trigo en 1996 para el Parque, y se aportaron 25 especies como nuevos registros al listado: *Bletia macristhmochila* Greenm., *B. neglecta* Sosa, *B. purpurata* A.Rich. et Galeotti, *B. purpurea* (Lam) DC., *Corallorhiza bulbosa* A.Rich. et Galeotti, *C. wisteriana* Conrad, *Deiregyne pyramidalis* (Lindl.) Burns-Bal., *Epidendrum magnoliae* Muhl., *Erycina hyalinobulbon* (Lex.) N.H.Williams et M.W.Chase, *Govenia capitata* Lindl., *Habenaria crassicornis* Lindl., *H. jaliscana* S.Watson, *H. novemfida* Lindl., *Laelia autumnalis* (Lex.) Lindl., *Malaxis brachyrrhynchos* (Rchb.f.) Ames, *M. salazari* Catling, *Mesadenus tenuissimus* (L.O.Williams) Garay, *Microthelys nutantiflora* (Schltr.) Garay, *Platanthera brevifolia* (Greene) Senghas, *Prosthechea linkiana* (Klotzsch) W.E.Higgins, *P. varicosa* (Bateman ex Lindl.) W.E.Higgins, *Schiedeella albovaginata* (C. Schweinf.) Burns-Bal., *S. confusa* (Garay) Espejo et López-Ferr., *S. llaveana* (Lindl.) Schltr. y *Stelis retusa* (Lex.) Pridgeon & M.W.Chase,

TABLA 1. Abundancia de especies de orquídeas en el PNIP y áreas de influencia.

Género	Cantidad
Bletia Ruiz & Pav.	4
Corallorhiza Châtel.	4
Deiregyne Schltr.	1
Dichromanthus Garay	2
Epidendrum L.	2
Erycina Lindl.	1
Funkiella Schltr.	1
Govenia Lindl.	2
Habenaria Willd.	3
Laelia Lindl.	1
Malaxis Sol. ex Sw.	5
Mesadenus Schltr.	1
Microthelys Garay	1
Platanthera Rich.	1
Prescottia Lindl.	1
Prosthechea Knowles & Westc.	2
Sarcoglottis C.Presl	1
Schiedeella Schltr.	4
Stelis Sw.	1
Stenorhynchos Rich. ex Spreng.	1

DISTRIBUCION Y ABUNDANCIA DE ORQUIDEAS. La distribución de orquídeas por Entidad Federativa en el PNIP y áreas de influencia (Fig. 1) se ubica con una cantidad de géneros y especies particulares, en Morelos se localizó la mayor cantidad; sin embargo, el menor número de especies fue en el estado de México. Las comunidades vegetales donde habitan preferentemente la mayoría de orquídeas son la de Pino-Encino y Pino (Fig. 2) y se sitúan entre los 2450 y 2750 m de elevación sobre el nivel del mar (Fig. 3).

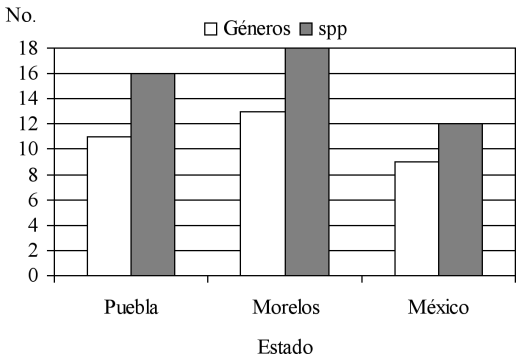

FIGURA 1. Distribución de orquídeas por entidad federativa en el PNIP y áreas de influencia.

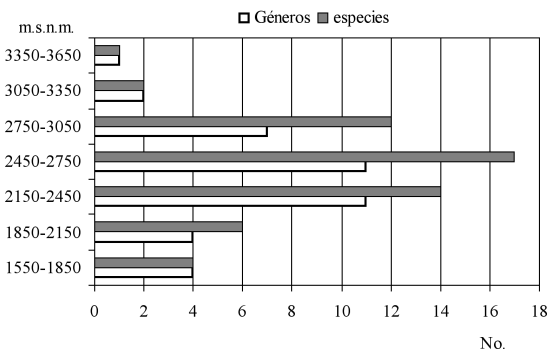

FIGURA 3. Abundancia de orquídeas por gradiente altitudinal en el PNIP y áreas de influencia.

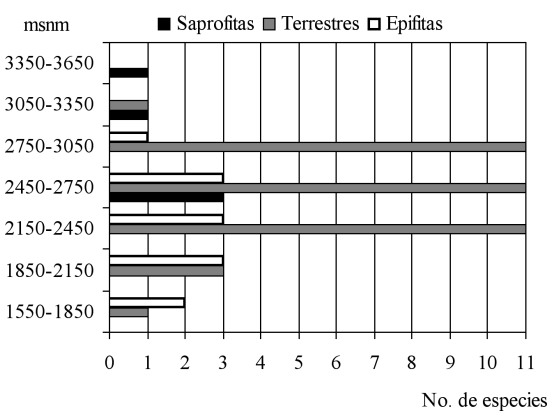

FIGURA 5. Abundancia de hábitos de crecimiento de orquídeas por rango altitudinal en el PNIP y áreas de influencia.

FORMAS DE VIDA Y FLORACION. Los hábitos de crecimiento saprofito, terrestre y epífito, están representados en las orquídeas localizadas en el Parque. Las de hábito terrestre son el tipo predominante en la zona de estudio con un 72% del total de especies y se distribuyen principalmente en Bosque de Pino-Encino y

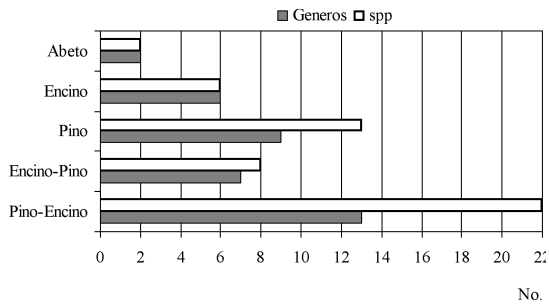

FIGURA 2. Abundancia de orquídeas por comunidad vegetal en el PNIP y áreas de influencia.

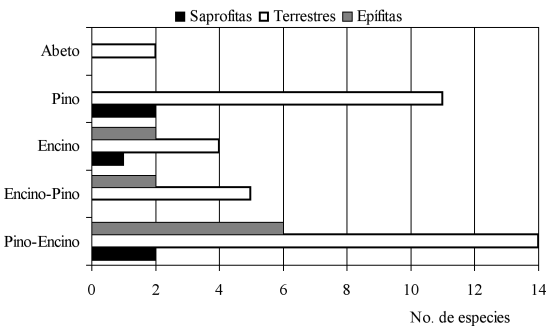

FIGURA 4. Abundancia de hábitos de crecimiento de orquídeas por comunidad vegetal en el PNIP y áreas de influencia.

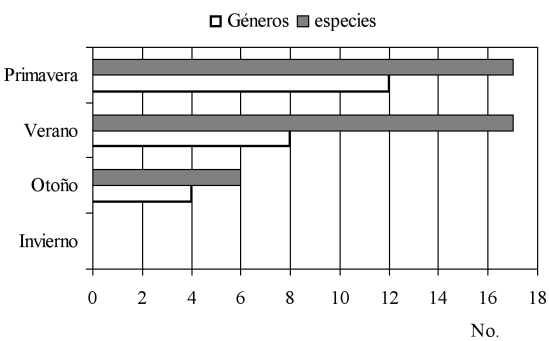

FIGURA 6. Época de floración de las orquídeas en el PNIP y áreas de influencia.

Pino (Fig. 4). De acuerdo a los gradientes altitudinales registrados se localizaron orquídeas terrestres desde los 1550 m hasta los 3350 m sobre el nivel del mar, las especies epifitas hasta cotas más bajas desde los 1550 a los 3050 m y las saprofitas en más altas, desde los 2450 hasta los 3650 (Fig. 5). De acuerdo a lo registrado, durante la realización del presente estudio, se localizaron especies terrestres principalmente en época de primavera y verano, todas ellas en floración, mientras que las epífitas fueron localizadas en

primavera otoño e invierno aún cuando no presentaron floración. Durante el invierno no se ha localizado ninguna especie en floración (Fig. 6).

Discusión

De la diversidad de orquídeas reportada por Chávez y Trigo en 1996, para el PNI-P y su área de influencia, solo cuatro géneros y 10 especies no se confirmaron. Después de 10 años de esa publicación y de cinco años de prospección en la zona de estudio se registraron 25 especies como nuevos registros, de las cuales dos son de hábito saprofito, 17 terrestre y seis epífito. En el estado de Puebla, que incluye las laderas del Este de los volcanes, se localizaron 16 especies de las cuales 8 son nuevos registros y concuerda con lo reportado por Chimal (1996) acerca de que faltaban especies por registrar en esta zona. La zona fisiográfica del PNIP y sus áreas de influencia permite que se establezca gran diversidad de especies terrestres, epífita y micotróficas; ya que de acuerdo con Hagsater *et al.* (2005), la zona presenta características tanto de las serranías del norte de México, cuya flora de orquídeas es poco diversa con especies terrestres y micotróficas, así como de las altas montañas del sur del país que permiten la existencia de una variada flora de orquídeas, donde las especies epífitas son tan numerosas como las terrestres. La Orchidaceae registrada en este estudio habita principalmente en Bosque de Encinos y Bosque de Coníferas, comunidades característicos de Bosques Templados Subhúmedos (Rzedowski 1978, Chávez y Trigo 1996, Hagsater *et al.* 2005). Mantener la diversidad de especies de orquídeas registradas en el Parque significa una gran responsabilidad, la alteración continua del hábitat, es un factor limitante para su localización, ya que diversos problemas ambientales y sociales amenazan seriamente esta riqueza natural. De aquí que se deba mantener la conservación de los sitios donde se encuentren la especies nativas y también la conservación *ex situ*.

Conclusiones

Se actualizó e incrementó el listado de orquídeas del PNIP y áreas de influencia con 39 especies incluidas en 20 géneros. Veinticinco especies y ocho géneros son nuevos registros. Las especies encontradas se distribuyen altitudinalmente desde los 1789 hasta los 3650 m. Predominan las orquídeas de habito terrestre y se distribuyen preferentemente en los bosques de pino o pino-encino.

Literatura Citada

Chávez, C.J.M. & B.N.Trigo (coords.). 1996. Programa de manejo para el Parque Nacional Iztaccíhuatl-Popocatépetl. UAM-Xochimilco, México, D.F. Departamento del Hombre y su Ambiente. Área de Ecología y Planeación de Recursos Naturales. 273 pp.

Chimal-Hernández, A., 1996. Vegetación. Pp. 77-87 *in*: C.J.M. Chávez & B.N. Trigo (coords.), Programa de manejo para el Parque Nacional Iztaccíhuatl-Popocatépetl. UAM-Xochimilco, México, D.F. Departamento del Hombre y su Ambiente. Área de Ecología y Planeación de Recursos Naturales.

CONANP, 2004. http://www.conanp.gob.mx/sinap/ (Consultada 21 de enero del 2007).

Hagsater E., M.Soto, G.Salazar, R.Jimenez, M.López & R.L. Dressler, 2005. Las Orquídeas de México. Instituto Chinoin, México.

Rzedowski, J., 1978. La Vegetación de México. Editorial Limusa. México.

Soto, M.A., 1988. Updated list of the orchids of Mexico. Orquídea (Méx.) 11: 273-276.

Soto, M.A. & E.Hagsater, 1990. Algunas ideas acerca de la conservación de las orquídeas mexicanas y un listado preliminar de los taxa amenazados. *In*: Áreas Naturales Protegidas en México y Especies en extinción. J.L.Camarillo & F.Rivera (Eds.) Unidad de Investigación ICSE, ENEP-Iztacala, UNAM.

Soto, M. A., E.Hagsater & G.A.Salazar, 2001. La Conservación de las Orquídeas de México. XV Congreso Mexicano de Botánica. Qro., Qro. México.

Vargas-Márquez, F., 1997. Parques Nacionales de México. Instituto Nacional de Ecología (INE), Secretaria de Medio Ambiente, Recursos Naturales y Pesca (SEMARNAP). D.F. México.

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AN ORCHID INVENTORY AND CONSERVATION PROJECT AT BOSQUE DE PAZ BIOLOGICAL RESERVE, UPPER RIO TORO VALLEY, ALAJUELA, COSTA RICA

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RESUMEN. El Jardín de Orquídeas de la reserva fue creado en el año 2000. Allí, las orquídeas caídas de los árboles del bosque son rescatadas, reubicadas y conservadas en árboles vivos (principalmente güitite, jaul y poró). Los objetivos del proyecto son: aumentar el conocimiento de la diversidad de orquídeas de la Cuenca del Río Toro mediante un inventario, respaldado por fotografías y material de herbario seco y en líquido, de las orquídeas rescatadas del bosque y cultivadas en el Jardín de la reserva y dar a conocer dicha reserva como ejemplo de ecoturismo educativo y sitio de gran importancia para la investigación orquideológica. El inventario se ha llevado a cabo desde junio del 2004. Se han identificado 47 géneros y 163 especies; 12 de éstas son endémicas de Costa Rica. En promedio, se observan 40 especies en floración cada mes. El hecho de que el Jardín de Orquídeas está situado junto a una reserva de vegetación natural, es una ventaja que puede aprovecharse para investigar sobre taxonomía y ecología de orquídeas de la región. Además de las opciones de investigación, Bosque de Paz realiza una importante labor en educación ambiental. Este inventario y la colección de herbario resultante son herramientas importantes para la investigación en orquideología. Consultar una colección de este tipo es de mucha utilidad tanto para estudios taxonómicos como ecológicos, en vista de que pocas veces se cuenta, como en este caso, con observaciones de plantas vivas, datos fenológicos, fotografías y material preservado, al mismo tiempo.

Orchids are among of the best-known and beloved plants, not only by scientists, but also by amateurs, and have a high commercial demand thanks to their beautiful, diverse and interesting flowers (Herrera 1998). It is the largest family of flowering plants in the world, with around 20,000 species (Dressler 1993). In Costa Rica there are around 1,400 registered species of orchids, but the knowledge of this family has grown a lot in recent years. Since 1993, around 20 new species have been described each year, and their classification is constantly changing because of molecular studies (Dressler 2003).

On the other hand, orchids are one of the most threatened groups of plants. Many species are considered endangered (Salazar 1996, Morales 2000). Most of the Orchidaceae family is included in the Appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which main objective is to regulate international trade to prevent species extinction because of this trade (or their overexploitation) (von Arx 1996).

Human activities have been causing, directly or indirectly, a decrease in orchid population sizes. The habitat alteration, including total destruction, modification and fragmentation, is the main problem for the conservation of the diversity. Most of the tropical orchids grow in primary forests. Some species are probably more tolerant to forest fragmentation than others; hence those less tolerant populations will decline more rapidly when habitats are altered. Another important threat is the illegal exploitation. A lot of plants are illegally collected from nature and sold (Salazar 1996, Morales 2000).

The main requirement for orchid conservation is therefore the maintenance of natural habitats (Light 2000, Catling 1996). The objective of *in situ* conservation is to allow species to be in the habitat where they belong and in the environment to which they are adapted (BGCI 1989). *Ex situ* conservation is the maintenance of organisms out of their natural habitat, for example in botanical gardens, field collections, and others, and its objective is to ensure the conserva-

tion of endangered species. *Ex situ* conservation is justifiable only when it is part of an integral conservation strategy (BGCI 1989).

The establishment of small natural reserves, sustained by private institutions, is an important strategy that complements the effort of the State to create and maintain the National Park System. In this way, a coordinated effort is made to conserve the Costa Rican natural and cultural patrimony (Fournier and Herrera 1979). Bosque de Paz is a private biological reserve located in the Central Volcanic Range. It has both primary and secondary forests, as well as grazing and in various states of reforestation (Kirby 2003). The Reserve was created with the objective of protecting the flora and fauna of the zone, and to create public awareness of the importance of conservation. The idea to relocate orchids for public viewing and scientific study began in the mid-90´s. After major storms with high winds and heavy rain occur, large number of branches and trees, full of epiphytic plants, fell across 20 km of trails in the Reserve. These orchids would die eventually due to low light and high humidity conditions. Fallen plants were subsequently rescued, and some of the orchid diversity of the area is now made accessible to visitors (Kirby 2003). In 1996 the Reserve had orchids relocated at eye level on trees along a 75 meter-long trail. In 2000, the Orchid Garden was created, at an elevation of about 1,550 meters above sea level, at 10°12.425' N latitude and 84°19.140' W longitude. The orchids are located on trees and live trunks.

To preserve orchid diversity, it is necessary to know which species exist, where they are located and basic aspects about their ecology and frequency in nature (Dressler 1996). Ideally, live plants in collections should be studied, but not every grower knows where their plants come from. In practice, one of the most common ways to obtain this kind of information is by visiting museums and herbariums, where dry material, sometimes complemented with flowers preserved in alcohol, can be found (Dressler 1996). Moreover, more elaborate surveys that give diversity, endemism, density and blooming data of the orchids present in a specific area, are even more valuable because they increase the knowledge of the distribution and ecology of the species, especially the rare ones (Soto 1996).

Surveys of plants present in National Parks, botanical gardens, as well as that of the biological preserves and private collections, are essential for the use of these places in conservation and research. Because of this, it is important to perform both taxonomic studies as sources of information about the species diversity in different places of the country, and ecological studies to know the habitat and the environmental conditions where the native orchids grow, as well as obtaining fundamental information on orchid biogeography (Kirby this volume). This study is believed to be the first comprehensive, multi-year collection, description and identification of orchids in the Central Volcanic Range in Costa Rica. The objective of this paper is to provide a species inventory of native orchids from the Río Toro Valley, Valverde Vega, Alajuela, as a baseline for conservation and starting point for orchid research in this region.

Methodology

An orchid survey at Bosque de Paz Biological Reserve has been in progress since June of 2004. Monthly field trips to the Reserve were made in order to sample blooming species. A herbarium collection was created and is currently maintained at the Reserve. Flowers were collected and preserved in liquid (55% alcohol, 5% glycerin and 40% water) as well. Every species was photographed and described using the checklist described by Kirby and Muñoz (this volume). Nomenclature follows that used by Dressler (2003). The blooming dates of every species were recorded and the identified plants were all labeled in the Orchid Garden.

Results

In the study period, 163 orchid species were observed in bloom and described, of which 12 species are endemics to Costa Rica. These were distributed into 47 genera. The genera with greatest number of species in the garden are: *Epidendrum* (24 spp.), *Pleurothallis* (23 spp.), *Maxillaria* (22 spp.) *Stelis* (10 spp.), *Lepanthes* (8 spp.), *Masdevallia* (7 spp.), *Prosthechea* (6 spp.), *Elleanthus* (5spp.), *Platystele* (4 spp.) and *Scaphyglottis* (4 spp.) (Table 1). On average, 40 (±11) species were observed in bloom each month. The months with more species in bloom were October, November and

TABLE 1. Orchid list of Bosque de Paz Biological Reserve.

Name	Field number
Acineta densa	04_98 (97)
Ada chlorops[a]	04_105
Barbosella dolichorhiza[a]	04_126
Brassia arcuigera	05_174
Chondrorhyncha picta[a]	04_100
Cryptocentrum calcaratum	04_104
Dichaea glauca[a]	04_147
Dichaea schlechteri E	04_128
Dichaea trichocarpa	04_75
Dracula carlueri	05_175
Elleanthus (2spp.)	06_220/ 06_238
Elleanthus cynarocephalus	04_77
Elleanthus glaucophyllus	05_173
Elleanthus lancifolius[a]	05_180
Encyclia ceratistes	04_82
Epidendrum (8 spp)	04_115[a]/ 04_156/ 05_177/ 05-187/ 06_210/ 06_216/ 06_221/ 06_236/
Epidendrum firmum	04_136
Epidendrum lacustre	b
Epidendrum lancilabium	05_204
Epidendrum laucheanum	04_93
Epidendrum myodes	05-184
Epidendrum palmense E	05_162
Epidendrum parkinsonianum	04_157
Epidendrum piliferum	04_91
Epidendrum platystigma E	05-181
Epidendrum radicans	04_154
Epidendrum sancti-ramoni[a]	05_161
Epidendrum subnutans[a] E	04_137(155)
Epidendrum summerhayesii	05-186
Epidendrum wercklei	b
Erythrodes killipii	06_215
Eurysyles standleyi E	07_243
Gongora horichiana	04_112
Govenia quadriplicata	06_224
Houlletia tigrina	06_231
Leochilus tricuspidatus	04_130
Lepanthes (7spp.)	05_158/ 05_164/ 05_190/ 06_207/ 06_214/ 06_217/ 06_219/
Lepanthes crossota	04_114
Lockhartia hercodonta	06_241
Lockhartia oerstedii	04_102
Lockhartia oerstedii[a]	05_178
Lycaste macrophylla	04_99
Masdevallia sp.	06_240
Masdevallia calura E	04_80
Masdevallia chontalensis	05_205
Masdevallia nidifica	06_212
Masdevallia picturata	06_234
Masdevallia pygmaea	06_228
Masdevallia striatella[a]	04_131
Maxillaria (5 spp.)	04_96[a]/ 05_189/ 06_213/ 06_227/ 06_237/
Maxillaria angustisegmenta[a]	04_110
Maxillaria biolleyi	04_146
Maxillaria bradeorum	05_163
Maxillaria brevilabia	04_148
Maxillaria cucullata	04_140
Maxillaria dendrobioides[a]	04_141
Maxillaria flava	06_235
Maxillaria fulgens	04_74
Maxillaria inaudita	04_145
Maxillaria microphyton[a] E	05_176
Maxillaria nasuta	04_123
Maxillaria porrecta	04_125
Maxillaria pseudoneglecta[a]	04_127
Maxillaria ringens	04_124
Maxillaria sigmoidea	06_239
Maxillaria umbratilis	06_208
Maxillaria wercklei E	05_192
Miltoniopsis warscewiczii	04_132
Oerstedella endresii	04_143
Oerstedella exasperata	04_70
Oerstedella intermixta E	04_107
Oncidium	04_152
Oncidium bracteatum	04_81 (83)
Oncidium klotzschianum	04_129
Oncidium panduriforme[a]	04_85
Osmoglossum egertonii	04_134
Otoglossum chiriquense	06_232
Phragmipedium longifolium[a]	04_92
Platystele compacta	04_89
Platystele lancilabris[a] E	05_166
Platystele oxyglossa[a]	04_103
Platystele propinqua[a] E	04_113
Pleurothallis (10 spp.)	04_101[a]/04_116[a]/ 04_120/ 04_139/ 04_153/ 05-188/ 06_211/ 06_218/ 06_230/ 06_242/
Pleurothallis amparoana[a]	05_171

E = Endemic species to Costa Rica. [a] = Samples with duplicates in the Herbarium of the University of Costa Rica. [b] = Not collected plants, just identified in the Orchid Garden.

TABLE 1 (continuation). Orchid list of Bosque de Paz Biological Reserve.

Name	Field number	Name	Field number
Pleurothallis cardiothallis[a]	04_108	*Scaphyglottis pachybulbon*[a]	04_149
Pleurothallis costaricensis[a]	05_165	*Scaphyglottis pulchella*	04_84
Pleurothallis dentipetala	05_203	*Scaphyglottis sigmoidea*[a]	04_86
Pleurothallis eumecocaulon	04_133	*Sigmatostalix picta*	04_90
Pleurothallis johnsonii	04_117	*Sobralia amabilis*	06_233
Pleurothallis palliolata	05_202	*Sobralia leucoxantha*	06_225
Pleurothallis phyllocardioides [a]	04_118	*Solenocentrum costaricense*	04_76
Pleurothallis pompalis[a]	04_88	*Stanhopea costaricensis*	06_226
Pleurothallis ramonensis E	04_87	*Stelis* (8 spp).	04_142/ 04_144/ 05_167[a]/ 05_170/ 05_174/ 05-182/ 05-183/ 05-185
Pleurothallis ruscifolia	04_72		
Pleurothallis tonduzii[a]	04_95		
Prosthechea sp.	06_206	*Stelis gracilis*[a]	04_109
Prosthechea brassavolae[a]	04_106	*Stelis ovatilabia*	04_119
Prosthechea campylostalix[a]	05_168	*Systeloglossum costaricense*	06_229
Prosthechea ionocentra	04_94	*Telipogon biolleyi*	04_71
Prosthechea pseudopygmaea	04_138	*Trichopilia marginata*	06_209
Prosthechea vespa	05_193	*Trichopilia suavis*	04_122
Restrepia muscifera[a]	04_135	*Trichosalpinx* sp.	06_216
Restrepia trichoglossa	04_121	*Trichosalpinx memor*	05_159
Rossioglossum schlieperianum	05_179	*Trichosalpinx memor*	05_160
Salpistele brunnea	05_191	*Warszewiczella discolor*	04_150
Scaphosepalum anchoriferum	04_79	*Xylobium elongatum*	04_111
Scaphyglottis densa[a]	05_169	*Xylobium sulfurinum*	04_73

E = Endemic species to Costa Rica. [a] = Samples with duplicates in the Herbarium of the University of Costa Rica. [b] = Not collected plants, just identified in the Orchid Garden.

December (Fig. 1). Dried herbarium sheets were prepared from plants and flowers of 149 species and flowers from 139 species were preserved by pickling. Duplicates of 36 species were deposited in the Herbarium of the University of Costa Rica (USJ).

Discussion

Having more than 160 species registered so far, with at least 12 being endemic, Bosque de Paz can now be recognized as a key site for *in situ* conservation of orchids in Costa Rica. With an area of 2000 hectares and with elevations ranging between 1,300 and 2,450 meters, the Reserve brings a big, little fragmented area, with modest human impact and with several microhabitats that support the existence, reproduction and other natural biological processes of an important number of orchids.

Bosque de Paz is a natural reserve, which has had success in the conservation of a group of plants as vulnerable as orchids. This also reflects success in the conservation of other plant families present in the zone. Moreover, the Orchid Garden could be considered a potential bank of germoplasm in the field (BGCI 1989). Field collections like this are better than conventional ones, because they have very similar characteristics to the natural habitat. The relocated plants have similar elevation, rainfall, temperature and pollinators where they were found. According to BGCI (1989) such collections should be the main *ex situ* conservation strategy. The Garden is located just next to an important natural forest, which is an advantage that could be further exploited for the taxonomic, ecologic and biogeografic studies of the region. Since it is the first multi-year orchid survey in the Central Volcanic Range, it is a starting point for comparisons with other montane cloud-forest environments in Costa Rica and elsewhere in Latin America (see Kirby, this volume).

Furthermore, one of the most important roles of

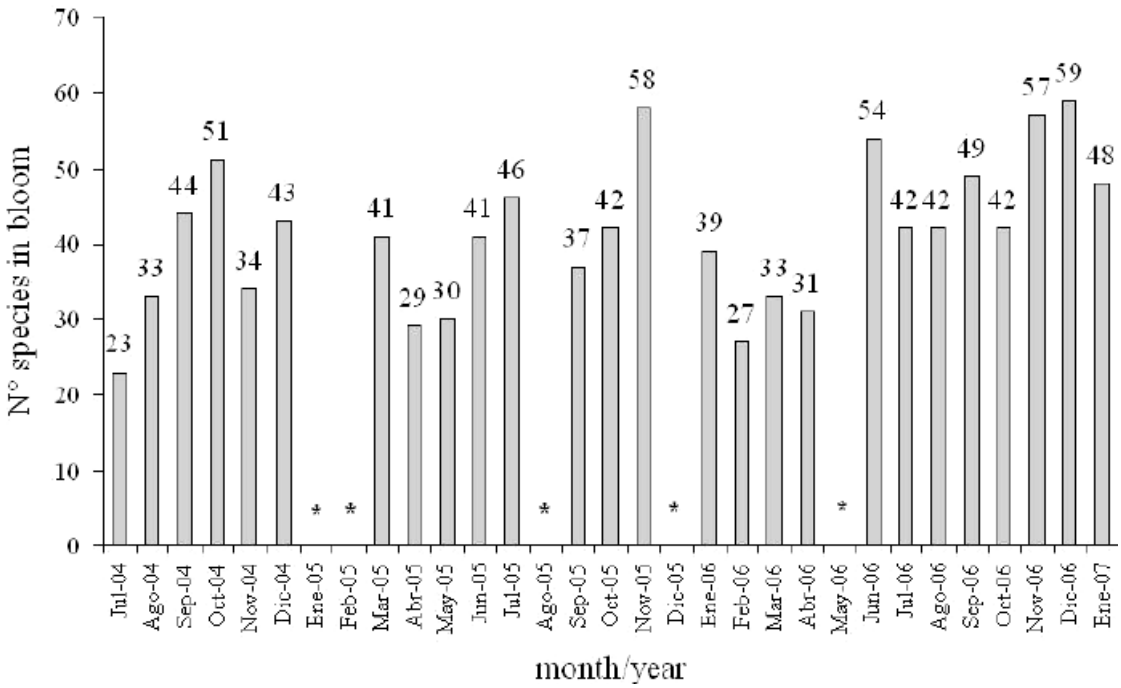

Figure 1. Number of species observed in bloom from July 2004 to January 2007 in the Orchid Garden of Bosque de Paz Reserve. *Data not collected.

natural preserves is to educate the people who visit them (Head and Lauer 1996). The creation of an orchid garden is therefore important for environmental education of both national and foreign tourists, because thanks to it, there is a great number and diversity of blooming orchid species that can be easily seen in the garden throughout the year, and are difficult to observe in their natural habitat. This educational opportunity helps to create consciousness about Costa Rica's natural richness, the enormous orchid diversity, the problems that make their conservation difficult, and that everybody can do something for their protection, such as the simple action of not taking them from their natural habitats.

Orchid surveys such this one are also valuable tools for orchid scientists. High-resolution digital and printed photographs, high quality herbarium samples, both dry and pickled specimens, with duplicates in the Herbarium of the University of Costa Rica (USJ) are provided. Access to a collection like this one could be very useful to researchers for taxonomic studies, for which there is limited preserved material, especially for those less conspicuous and rare species. Accurate species identifications also will be useful for population studies and orchid biogeography.

To conclude, Bosque de Paz Biological Reserve reflects the great orchid diversity of the area. Moreover, the reserve's Orchid Garden is a very important place for conservation, research and environmental education in several fields, with an obvious emphasis in orchideology.

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LITERATURE CITED

BGCI (Botanical Gardens Conservation International). 1989. La estrategia de los Jardines Botánicos para la Conservación. BGCI, WWR y UICN. Suiza. 51 p.

Catling, P.M. 1996. Conservation Strategy: *In situ* conservation. Pp. 15-23. *In:* E. Hágsater & V. Dumont (eds.). Orchids – Status Survey and Conservation Action Plan. IUCN. Gland Switzerland and Cambridge, UK.

Dressler, R.L. 1993. Phylogeny and Classification of the Orchid Family. Dioscorides Press. Hong Kong. 314 p.

Dressler, R.L. 1996. The problems associated with botanical sampling and study. P. 34 *in:* E. Hágsater & V. Dumont (eds.). Orchids- Status Survey and Conservation Action Plan. IUCN. Gland, Switzerland and Cambridge, UK.

Dressler, R.L. 2003. Orchidaceae. *In*: Hammel, B. E., Grayum, M. H., Herrera, C. & Zamora, N. (eds.). Manual de Plantas de Costa Rica, vol III: Monocotiledóneas (Orchidaceae-Zingiberaceae). Missouri Bot. Gard. INBio. Museo Nacional de Costa Rica. Pp. 1-595.

Fournier, L.A. & M.E. Herrera. 1979. Importancia científica, económica y cultural de un sistema de pequeñas reservas naturales en Costa Rica. Agron. Costarr. 3(1): 53-55.

Head, C. & A. Lauer. 1996. Conservation Strategy: Education. Pp. 46-47 *in:* E. Hágsater & V. Dumont (eds.). Orchids – Status Survey and Conservation Action Plan. IUCN. Gland Switzerland and Cambridge, UK.

Herrera, A. 1998. Factibilidad técnica y financiera para la producción *in vitro* de orquídeas (*Masdevallia calura y Masdevallia reichenbachiana*) en Monteverde, Puntarenas. Tesis de Licenciatura en Economía Agrícola, Universidad de Costa Rica, San José. 149 p.

Kirby, S.H. 2003. Neotropical orchid eco-tourism: educational experience of an orchid neophyte at The Bosque de Paz Biological Preserve, Central Volcanic Range, Costa Rica. Lankesteriana 7: 121-124.

Kirby, S.H. 2007. Geological Processes and Orchid Biogeography with Applications to Southeast Central America. Proceedings of the 3rd International Orchid Conservation Congress *in* Lankesteriana, this volume.

Kirby, S.H. & M. Muñoz. 2007. A form and checklist for the description of orchids in the field and laboratory work. Costa Rica. Proceedings of the 3rd International Orchid Conservation Congress *in* Lankesteriana, this volume.

Light, M.H.S. 2000. *In situ* orchid conservation: challenge and opportunity. Orchid Conserv. News 3: 4-5.

Morales, J.F. 2000. Orquídeas, cactus y bromelias del bosque seco. INBio. Santo Domingo de Heredia, Costa Rica. 162 p.

Salazar, G.A. 1996. Conservation Threats. Pp. 6-10 *in:* E. Hágsater & V. Dumont (eds.). Orchids- Status Survey and Conservation Action Plan. IUCN. Gland, Switzerland and Cambridge, UK.

Soto, M. 1996. Conservation Strategy: The importance of research. Pp. 33-38. *In:* E. Hágsater & V. Dumont (eds.). Orchids – Status Survey and Conservation Action Plan. IUCN. Gland, Switzerland and Cambridge, UK.

von Arx, B. 1996. Conservation Strategy: International Protection. Pp. 11-14. *In:* E. Hágsater & V. Dumont (eds.). Orchids – Status Survey and Conservation Action Plan. IUCN. Gland, Switzerland and Cambridge, UK.

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DISTRIBUCIÓN DE POBLACIONES SILVESTRES Y DESCRIPCIÓN DEL HÁBITAT DE *PHRAGMIPEDIUM* EN COSTA RICA

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PALABRAS CLAVE: *Phragmipedium*, slipper orchids, poblaciones silvestres, distribución, descripción de hábitat, orquídeas terrestres, Costa Rica

Las orquídeas de género *Phragmipedium* (Pfitz.) Rolfe pertenecen a la subfamilia Cypripedioideae y son comúnmente llamadas zapatillas o "slipper orchids" (Atwood 1984). Según Dressler (2003), en Costa Rica se encuentran dos especies: *P. humboldtii* (Warsz. *ex* Rchb.f.) J.T. Atwood & Dressler, la cual se encuentra también en México, Guatemala, Honduras, Nicaragua, Panamá y Perú (UNEP-WCMC 2004); y *P. longifolium* (Warsz. & Rchb.f.) Rolfe, que se distribuye en Costa Rica, Panamá, Colombia y Ecuador (UNEP-WCMC 2004).

El valor económico de estas plantas se debe a su gran belleza, la cual es el origen de su alta extracción ilegal en la naturaleza, reduciendo cada vez más el tamaño de sus poblaciones y llevándolas a peligro de extinción. Las dos especies reportadas para Costa Rica se encuentran en la lista roja de especies en peligro de extinción de la UICN (Unión Internacional para la Conservación de la Naturaleza) (Pupulin 2003). Además, este género está incluido en el Apéndice I de CITES (Convention on Internacional Trade in Endangered Species of Wild Fauna and Flora) (von Arx 1996).

La disponibilidad de información detallada es necesaria para poder tomar decisiones adecuadas sobre el manejo de las especies (Olson *et al.* 2005). Para saber el estado real de *Phragmipedium* en Costa Rica, es necesario conocer la distribución y las características de sus poblaciones silvestres. En este trabajo se presentan los resultados de una búsqueda sistemática de poblaciones de *Phragmipedium* en Costa Rica. Los objetivos del trabajo son establecer una distribución general de *Phragmipedium longifolium* y *P. humboldtii* en Costa Rica, basado en datos de herbario y de campo, y describir el hábitat donde se encuentran.

Metodología

Se obtuvieron datos de recolecta de ejemplares de *P. longifolium* y *P. humboldtii* depositados en el Herbario de la Universidad de Costa Rica (USJ), Herbario Nacional (CR) e Instituto Nacional de Biodiversidad (INBio) como base para iniciar la búsqueda de localidades conocidas de esta especie de orquídea en Costa Rica. Por otro lado, se contactaron biólogos, naturalistas, guardaparques, aficionados y coleccionistas que tuvieran conocimiento de localidades donde crecen las plantas. Se realizaron visitas a las localidades donde las plantas habían sido recolectadas u observadas. Las giras se realizaron durante 2005 y 2006. En cada sitio se recolectó material testigo que luego se depositó en la colección viva del Jardín Botánico Lankester. En cada población se tomaron las coordenadas geográficas con un GPS Garmin Map 76S. Se utilizó el programa ArcView GIS 3.3 para localizar en un mapa de Costa Rica las poblaciones reportadas en bases de datos de herbarios y las visitadas durante el estudio.

La descripción del hábitat se hizo según Zhan-Huo *et al.* (1999), se anotó la elevación, área aproximada que ocupa la población, cercanía a ríos, impacto de la actividad humana y presencia de brotes nuevos, flores y frutos en las plantas.

Resultados

EJEMPLARES DE HERBARIO. Del Herbario de la Universidad de Costa Rica (USJ) se obtuvieron datos de plantas de *P. longifolium* y *P. humboldtii* cultivadas en el Jardín Botánico Lankester, pero sin datos de procedencia. En dicho herbario, se obtuvo otro dato de *P. longifolium* cultivado en "La Finca el Trébol,

La Palma" pero sin coordenadas geográficas. En el Herbario Nacional se encuentran muestras de dos plantas de *P. humboldtii* de la zona sur del país. La primera, recolectada por Estrada A. *et al.* (2001) en cultivo en el Jardín Botánico Wilson, proveniente de Sabalito, San Vito de Coto Brus y la segunda recolectada cerca de la frontera con Panamá en 1923. Además, cinco ejemplares de *P. longifolium* provenientes de La Fortuna de San Carlos, San Ramón, Paraíso y Sarapiquí (cuadro 1). En el Herbario del INBio están registradas cinco muestras de *P. longifolium,* de las cuales dos son duplicados de las muestras del Herbario Nacional (cuadro 1). En el INBio no existen ejemplares de *P. humboldtii.*

GIRAS DE CAMPO. En total se visitaron 10 poblaciones de *P. longifolium* localizadas en las zonas de Venecia de San Carlos, Tilarán, Grecia, Paraíso y Sarapiquí (cuadro 1, figura 1). Los códigos del material testigo de cada población depositados en el Jardín Botánico Lankester se muestran en el cuadro 1. Durante el periodo de estudio no fue posible localizar alguna población de *P. humboldtii*.

DESCRIPCIÓN DEL HABITAT. La mayoría de las poblaciones visitadas se encuentran entre 950 y 1255 msnm, excepto las de La Virgen de Sarapiquí, Reserva Gavilán Blanco y Reserva Rara Avis que se encuentran en zonas más bajas (cuadro 1). Según las zonas de vida establecidas por Holdridge (1967) todas las poblaciones, tanto las localizadas en el campo como los registros de herbario, se encontraron en el bosque muy húmedo tropical transición a premontano y bosque pluvial premontano, con excepción de las poblaciones de La Virgen, que se encontró en bosque muy húmedo tropical, y de Tilarán I, que se localizó en bosque muy húmedo premontano transición a pluvial (figura 1).

Todas las poblaciones se encontraron formando parches pequeños de 9-2500 m^2, solamente las poblaciones del Proyecto Hidroeléctrico Toro II del Instituto Costarricense de Electricidad (ICE), Tilarán II y Río Cuarto poseían áreas más grandes (cuadro 1). Los parches de plantas más pequeños, localizados en La Virgen y en la Reserva Gavilán Blanco, constaban de únicamente 4-6 plantas cada una, sin embargo, éstas se encontraban en buen estado, con brotes nuevos, flores e incluso cápsulas.

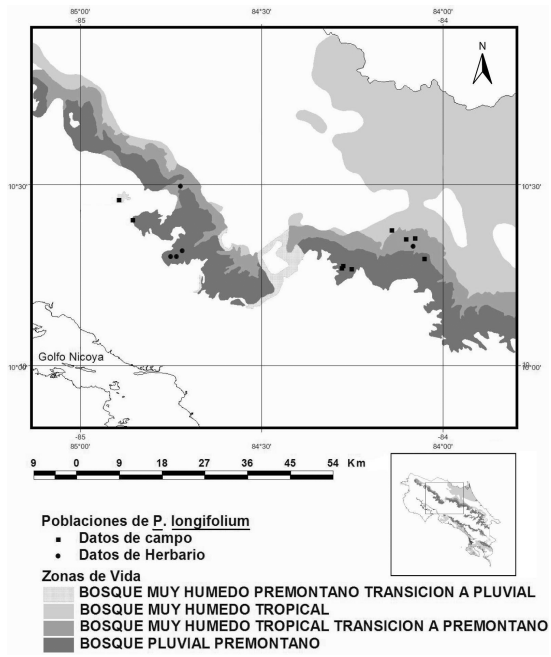

FIGURA 1. Mapa de distribución de *P. longifolium* en Costa Rica según las zonas de vida establecidas por Holdridge (1967).

Las plantas de *Phragmipedium* de la Reserva Gavilán Blanco, Rara Avis, Aguas Silvestres y La Virgen se encontraron creciendo en zonas de poca cobertura vegetal, sobre rocas grandes dentro de ríos de poco caudal en estación seca (entre 5 y 15 m de ancho y alrededor de 1-1.5 m de profundidad), ubicadas al lado contrario de la corriente de agua, o en rocas a la orilla de los mismos. Otras plantas crecen en paredones ubicados a la orilla de caminos, tal es el caso de las poblaciones encontradas en Venecia, Tilarán I y Río Cuarto. En las dos últimas los paredones estaban adyacentes a cauces de ríos pequeños o quebradas. Por otro lado, en Toro II y Paraíso, los paredones están continuos a cataratas y el acceso es limitado para el hombre. La población más grande encontrada fue la de Tilarán II (cuadro 1), en la cual las plantas crecen en un potrero sin sombra y a la orilla de una carretera. Las poblaciones de Tilarán son las dos únicas donde las plantas no se encontraron en parches adyacentes a alguna quebrada o río. Las plantas de todas las poblaciones encontradas poseían flores y brotes nuevos y no presentaban signos visibles de enfermedades causadas por hongos o bacterias. No se encontraron plantas de *P. longifolium* epífitas.

CUADRO 1. Datos de recolecta de *P. longifolium* de las muestras depositadas en el Herbario Nacional e INBio y de las muestras testigo, de las poblaciones visitadas, depositadas en el Jardín Botánico Lankester (JBL).

Población	Ubicación	Altitud (msnm)	Área (m2)	Fecha recolecta	Recolector (nº de recolecta)	Herbario	N° JBL	Zona protegida	Lugar donde crecen
Monteverde	San Ramón	800		1990	*Bello 1987*	CR / INB	-	X	Roca de río
Monteverde	San Ramón	900		1987	*Haber 7165* y *7886*	CR	-	X	Roca de río
Braulio Carrillo	Sarapiquí	500-600			*Zumbado 15*	CR / INB	-		Borde de bosque
Cachí	Paraíso	1450		1969	sin datos recolecta	CR	-		Sin dato
La Fortuna	San Carlos	1025		1978	*Hamel s. n.º*	CR	-		Sin dato
Arenal	San Carlos	500-600		1994	*Lepiz et al. 119*	INB	-		Terrestre en sotobosque
Monteverde	San Ramón	850		1897	*Haber 7886*	INB	-	X	Roca de río
La Virgen	Sarapiquí	283	50	2004		-	-		Roca de río
Venecia	San Carlos	1084	500	2005	*Warner 9*	-	11599		Paredón
Proyecto Hidroeléctrico Toro II	San Carlos	1081	10000	2005	*Warner 5*	-	11606	X	Paredón
Centro Biológico Aguas Silvestres	Sarapiquí	1081	1600	2005	*Warner 16*	-	11737	X	Roca de río
Reserva Biológica Gavilán Blanco	Sarapiquí	670	9	2005	*Warner 22*	-	11597	X	Roca de río
Reserva Rara Avis	Sarapiquí	724	1500	2005	*Warner 42*	-	12064	X	Roca de río
Tilarán I	Tilarán	1255	2500	2006	*Warner 84*	-	-		Paredón
Tilarán II	Tilarán	951	60000	2006	*Warner 85*	-	-		Potrero
Rio Cuarto	Grecia	1099	60000	2006		-	-		Paredón
Paraíso	Paraíso		2400	2005	*Warner 78*	-	12746		Paredón

Discusión

Las colecciones de herbario son una fuente importante de datos de distribución de las especies de plantas (Jones *et al.* 1997). Los datos obtenidos de los herbarios consultados fueron una base muy importante para el inicio de la búsqueda de las poblaciones silvestres de *Phragmipedium* en Costa Rica. Se encontraron poblaciones cercanas a sitios de recolecta reportados en los herbarios, como es el caso de la región de Sarapiquí, y alrededores de Monteverde (Tilarán, San Ramón, La Fortuna de San Carlos) y Paraíso. Además, se hallaron poblaciones en zonas que no estaban reportadas anteriormente en los registros de herbario (Venecia, Toro II y Río Cuarto), lo cual es una contribución importante al conocimiento de la distribución geográfica de este género en Costa Rica. Por otro lado, los tipos de sustrato reportados en los herbarios concuerdan con los encontrados en el campo.

Es importante notar que la mayoría de las poblaciones de *P. longifolium* se encontraron creciendo en zonas generalmente asociadas a fuentes de agua o expuestas a mucha humedad, con escasa o ninguna cobertura vegetal y con diferentes niveles de alteración humana o natural, como derrumbes o crecidas de ríos. Las características de las poblaciones observadas indican que las plantas de *P. longifolium* son capaces de colonizar ambientes alterados, expuestos a la luz y la humedad, donde las semillas dispersadas por el viento encuentran las condiciones necesarias para germinar y desarrollarse. El caso más notorio de plantas creciendo en una zona con alto impacto humano es la de Tilarán II, donde las plantas crecen en un potrero, sin sombra y a la orilla de un camino asfaltado, y sin embargo, fue la población con mayor cantidad de plantas y área ocupada.

El hecho de que las poblaciones estén conformadas por pequeños parches, donde las plantas están muy cercanas unas de otras, y que se encuentren en lugares alterados, con fácil acceso humano, hacen muy vulnerables a estas poblaciones silvestres. Por otro lado, algunas poblaciones se encuentran en áreas protegidas, como las reservas privadas Aguas Silvestres, Gavilán Blanco y Rara Avis y en sitios como la Planta Hidroeléctrica Toro II del ICE, donde el acceso a particulares es limitado, lo cual brinda mayor protección a esta orquídea en la zona.

El presente trabajo brinda información general de las características del hábitat y de la distribución geográfica de *P. longifolium* en Costa Rica. Aunque se visitaron todas las localidades donde se conoce la presencia de plantas de este género, la búsqueda no fue exhaustiva por lo que seguramente existen otras poblaciones viviendo en condiciones similares a las descritas anteriormente. Por otro lado, la información disponible acerca *P. humboldtii* es muy escasa y la ubicación de poblaciones en la zona sur de Costa Rica resulta difícil. En el futuro se pueden utilizar bases de datos geo-referenciados y programas de Sistemas de Información Geográfica para elaboración de mapas que permitan identificar y limitar nuevas áreas de búsqueda de estas especies.

AGRADECIMIENTOS.Se agradece al Instituto Costarricense de Electricidad, a Luis Diego Gómez, Orlando Vargas, Amos Bien, Eladio Cruz, Álvaro Salazar, Ernesto Carman y Vinicio Porras por su valiosa ayuda en la localización y acceso a las poblaciones silvestres de *P. longifolium* visitadas durante este estudio, así como al Ministerio de Ambiente y Energía por los permisos para recolectar material. Este trabajo fue financiado parcialmente por la Vicerrectoría de Investigación de la Universidad de Costa Rica, en el marco del proyecto: "Evaluación de la variabilidad genética de poblaciones silvestres y cultivo *in vitro* de *Phragmipedium* (Orchidaceae) en Costa Rica" (814-A6-107).

LITERATURA CITADA

Atwood, J.T. 1984. The relationship of the slipper orchids (Subfamily Cipripedioide, Orchidaceae). Selbyana 7: 129-229.

Dressler, R.L. 2003. Orchidaceae. *In*: Hammel, B. E., Grayum, M. H., Herrera, C. & Zamora, N. (eds.). Manual de Plantas de Costa Rica, vol III: Monocotiledóneas (Orchidaceae-Zingiberaceae). Missouri Bot. Gard. INBio. Museo Nacional de Costa Rica. Pp. 1-595.

Holdridge, L. 1967. Life Zone Ecology. Tropical Science Center, Costa Rica. 89 p.

Jones, P.G., S.E. Beebe & J. Tohme. 1997. The use of geographical information systems in biodiversity exploration and conservation. Biodiversity and Conservation 6: 974-958.

Olson, M.E., J.A. Lomeli & N.V. Cacho. 2005. Extinction thread in the *Pedilanthus* clade (*Euphorbia,* Euphorbiaceae), with special reference to the recently rediscovered *E. conzattii* (*P. pulchellus*). Am. J. Bot. 92(4): 43-49.

Pupulin, F. 2003. Orchidaceae de Costa Rica. Jardín Botánico Lankester. Universidad de Costa Rica. Costa

Rica. 28 pp.
UNEP-WCMC (United Nations Environment Programme - World Conservation Monitoring Centre). 2004. UNEP-WCMC Species Database: CITES-Listed Species. (consultada el 30 de setiembre 2004, http://sea.unep-wcmc.org/isdb/CITES/Taxonomy/tax-species-result.cfm?Genus=Phragmipedium
von Arx, B. 1996. Conservation Strategy: Internacional Protection, pp11-14. *In:* E. Hágsater & V. Dumont (eds.). Orchids - Status Survey and Conservation Action Plan. IUCN. Gland Switzerland and Cambridge, UK.
Zhan-Huo, T., L. Yi-Bo, P.J. Criba, N. McGough, G. Siu, & L. Chau. 1999. A preliminary report on the population size, ecology, and conservation status of some *Paphiopedilum* species (Orchidaceae) in southwest China. Lindleyana 14(1): 12-23.

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Jorge Warner es biólogo con estudios de posgrado en la Universidad de Costa Rica. Trabaja con el Jardín Botánico Lankester desde 1991. Sus áreas de trabajo son cultivo *in vitro* de plantas en peligro de extinción y conservación *in situ*.

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ORCHIDS OF A REGENERATED TROPICAL DRY FOREST IN THE CALI RIVER WATERSHED, MUNICIPALITY OF CALI, COLOMBIA

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RESUMEN: El bosque seco tropical regenerado en la cuenca media del Río Cali forma un corredor biológico de cerca de 100 hectáreas que conecta la ciudad de Cali con el Parque Natural Farallones de Cali. El Jardín Botánico de Cali, un espacio natural de bosque seco tropical regenerado de 12 hectáreas, forma parte de este corredor y su vegetación muestra una dominancia de especies pioneras de sucesión ecológica secundaria. Las especies que predominan son: "arrayán" (*Myrcia popayanensis*), Laurel Jigua (*Cynammomum triplinerve*), Sangregao (*Crotón gossypifolius*), Guácimo (*Guazuma ulmifolia*), Chiminango (*Pithecellobium dulce*) y Chagualo (*Clusia sp*). Un análisis preliminar de las orquídeas presentes en este corredor incluye especies que crecen bien en terrenos abiertos como *Cyrtopodium paniculatum* y *Catasetum ochraceum* y en afloramientos rocosos como *Epidendrum xanthinum*, *Schomburgkia y Sobralia.* Las especies epífitas del JB incluyen *Dimerandra emarginata*, *Catasetum tabulare*, *Encyclia ceratistes*, *Encyclia sp*), *Bulbophyllum meridense*, *Cladobium, Epidendrum* (3 spp), *Maxillaria* (2 spp), *Lepanthes* y *Oncidium cartaginensis*. Hay dos especies de vainillas que son propiamente especies trepadoras. Las especies típicamente terrestres incluyen los siguientes géneros: (*Oeceoclades, Cleistes, Galeandra, Pelexia y Spiranthes.* En un bosque seco tropical de condiciones similares en la cuenca del Río Claro se encontró una especie de *Coryanthes* posiblemente nueva. La vegetación presente en el corredor biológico y el JBC es una regeneración de los últimos 70 años. El área había sido impactada severamente por procesos de agricultura extensiva, ganadería, proyectos viales y por incendios forestales. Las especies nativas de árboles así como las orquídeas presentes actualmente conforman un banco de germoplasma de gran valor particularmente desde la óptica de la restauración ecológica del bosque seco tropical en laderas andinas. Esta flora de orquídeas es precisamente, la misma que alguna vez existía en los bosques donde hoy la ciudad de Cali se extiende y por tanto representa una ventana del pasado y un enorme potencial educativo para las generaciones presentes.

KEY WORDS: orchids, restoration, conservation, systematics

Introduction

The Tropical dry Forest (Bs-T) is a vegetal formation with continuous forest cover between 0-1,000 m in altitude and temperatures above 24 C and average annual rainfall between 700 and 2,000mm, with one or two dry periods per year (Espinal 1985; Murphy & Lugo 1986, InstitutoAlexander von Humboldt 1998). The Tropical dry Forest represents about 50% of the forested areas of Central America and 22% of South America (Murphy and Lugo, 1986). In Colombia this formation is found in the Caribbean region and in the interandean valleys of the rivers Magdalena and Cauca in an area which presumably covered about 8,146,000 hectares (Espinal and Montenegro, 1977).

The Tropical dry Forest is one of the most threatened ecosystems of the Neotropics (Janzen, 1987). In Colombia it is one of the most degraded and fragmented, with estimates of present total cover of less than 1.5% of the original cover (Etter, 1993). Of this total the greatest proportion is found in the arid peri-caribbean belt with more than 6 million hectares and the NorAndean -Chocó-Magdalena province with about one million hectares (Hernández et al. 1992, Espinal and Montenegro 1977). The dry forest of the upper Cauca river valley, the main tributary of the Magdalena river, originally covered about 300,000

hectares in the Department of Valle del Cauca. Presently, the dry forest of this region has practically disappeared to the advance of sugarcane cultivation, the major economic crop of the State. It is estimated that the cover of this formation in the Cauca Valley is less than 3,000 hectares with documented reductions of 66% between 1957 and 1986 (CVC 1994). Only a few forest relicts remain in the flat portion of the Cauca river valley, all below 12 hectares each. The situation is only slightly less dramatic along the piedmont areas of the Central and Western Andean ranges where a few remnants and regenerated forests exist. The lower and middle portion of the Cali river presents a sizeable sample of the tropical dry forest formation.

The regenerated forest of the middle portion of the Cali River still guards some orchid treasures and is important for conservation purposes (Orejuela 2005, 2006). Without a previous study of the orchids of this watershed, it seemed appropriate to look at the orchids present today after some 70 years of advance of the regeneration process and to attempt to discover the original orchid flora of the local piedmont area in the municipality of Cali. This study presents a composite picture of the orchids of the lower Eastern Andean slopes as the Andes merges with the Cauca river valley. The orchids of this region are typically species of ecological succession. As the forest matures the orchid flora will increase in number of species and possibly also in terms of density of individuals. For now, it is of interest to see a diversified array of species.

General obiective

To determine the composition and growth mode of the orchids present in the regenerated tropical dry forest formation along the middle portion of the Cali river basin with the purpose of conserving the species present, to reintroduce those species which were possibly present in the watershed and to enrich the orchid species collection at the Cali Botanical Garden.

Specific Objectives

To determine the species composition and the growth mode of the orchid species found in the regenerated tropical dry forest formation in the biological corridor of the middle portion of the Cali river basin. To enrich the vegetation and area of the CBG with species of orchids found in the surrounding areas of the garden and in the "sister" watersheds of the Cali river basin.

To determine the potential of the forest remnant of the Cali Botanic Garden to serve as a source of germplasm to undertake restoration processes along the middle sector of the Cali river basin and in the city of Cali.

To design a community conservation education strategy about the orchids (and the associated animal species) of the Cali river watershed and of the Botanical Garden.

Methods

COLLECTION, IDENTIFICATION AND TABULATION OF THE ORCHID SPECIES. The characterization of the vegetation was developed in three stages: The collection of plants, the identification and the tabulation of the species found. The area inventoried covered approximately 45 hectares of forest including the totality of the area of the botanical garden and a forest of 35 hectares under protection by the Utilities Company EPSA. In addition, selected visits were made to similar regenerated and relictual forests of several "sister" watersheds like Rio Claro, Jamundí and Pance. These watersheds originate in the high Andean mountain of the Farallones National Park and descend rapidly to tribute waters into the Cauca river. The orchids collected were assigned to the following growth category: Terrestrial, lithophitic, climber and epiphyte.

COMPARATIVE ANALYSIS OF THE ORCHID FLORA OF THE VARIOUS WATERSHED to establish the breath of species present along the Andean piedmont area adjacent to the Cauca river valley. The "mother" list of potential species which is generated serves as a germplasm bank which could be used for reintroduction purposes in the Cali river basin and in the entire piedmont area of the Municipality.

REINTRODUCTION OF SELECTED SPECIES. A protocol was designed to establish the species most suited for reintroduction in the regenerated forest. A species photographic catalogue was made of the species of the watershed.

A CONSERVATION EDUCATION PROGRAM was developed to use orchids as indicator species of the benefits of

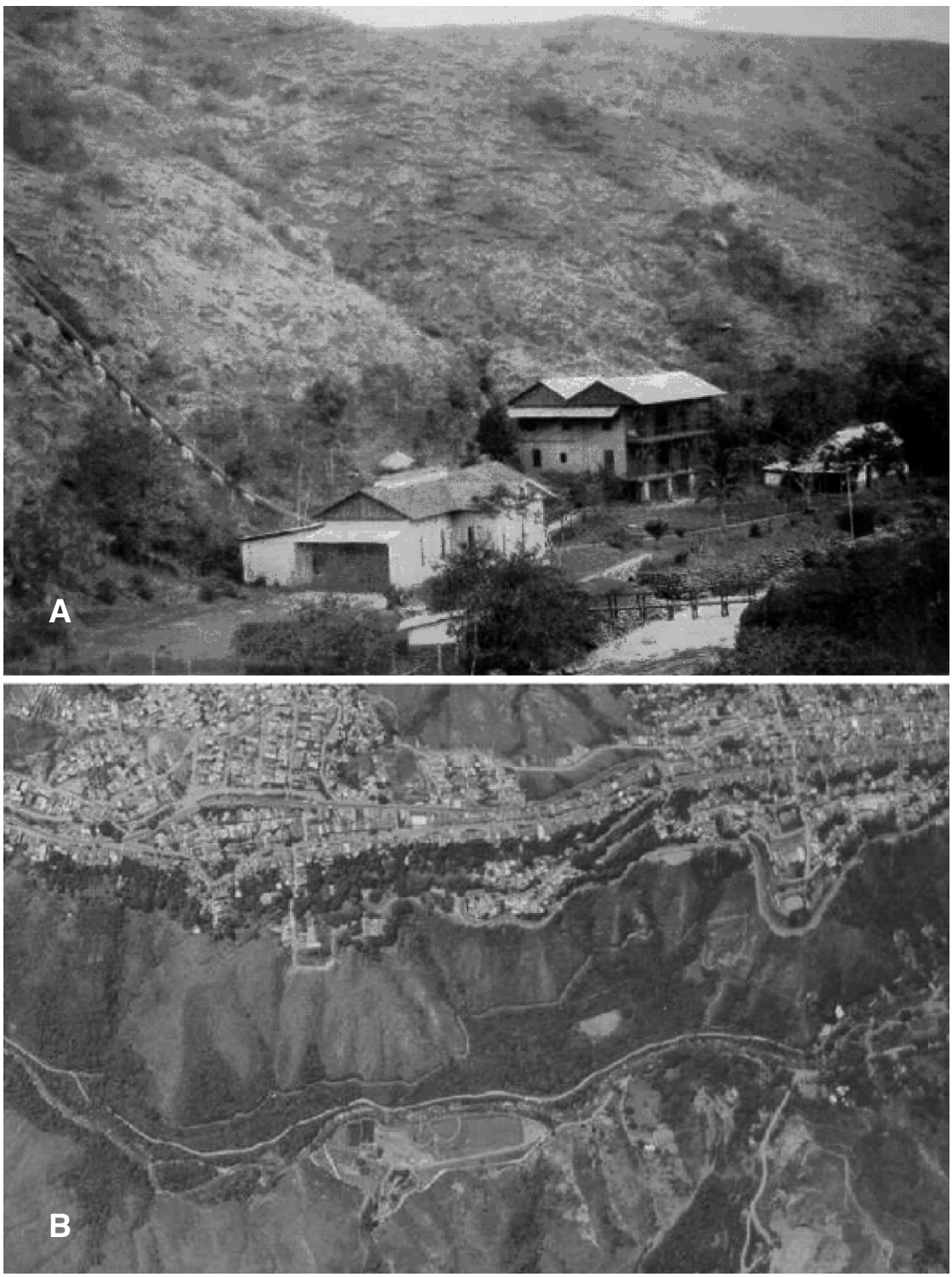


FIGURE 1. A - State of the habitat in the hillsides surrounding the first power plant of Cali, 1910. B - Location of the Cali Botanical garden and the biological corridor of the Cali river.

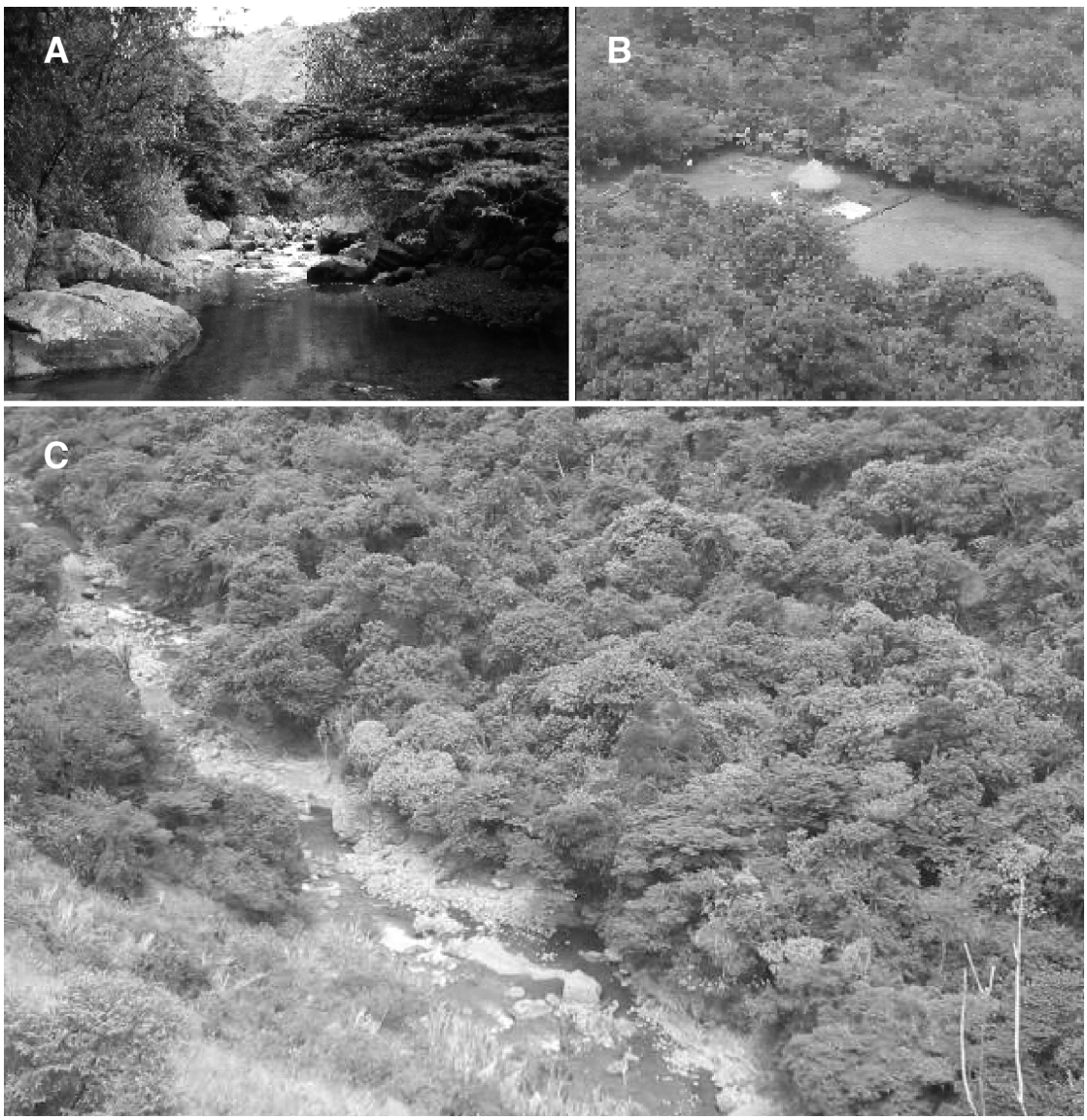


FIGURE 2. A - Cali River. B - Botanic Garden. C - Cali River and Tropical dry Forest. The totality of the flora of the botanical garden constitutes a germplasm bank of native pioneering species ideal to advance reforestation processes in the interandean river valleys. About 20-25 tree species were identified as promissory for ecological restoration and enrichment processes along Andean hillsides.

an assisted regeneration process. The elements of the strategy include: viewing of prepared video of the orchids of the Cali area; Jinkana observation games to spot and identify the orchids which enrich the Botanic Garden orchid collection; student visits to the Garden´s orchidarium, and to the orchid stand along the interpretive nature trail; preparation of orchid herbaria by students of the local schools.

Results

HISTORY OF THE REGENERATION PROCESS**.** By 1910, the inauguration date of the first hydroelectrical power plant of Cali, the native vegetation had been totally eliminated. A combination of reason explain this forest conversion: large demand of wood charcoal by the 25,000 inhabitants of Cali; removal of native veg-

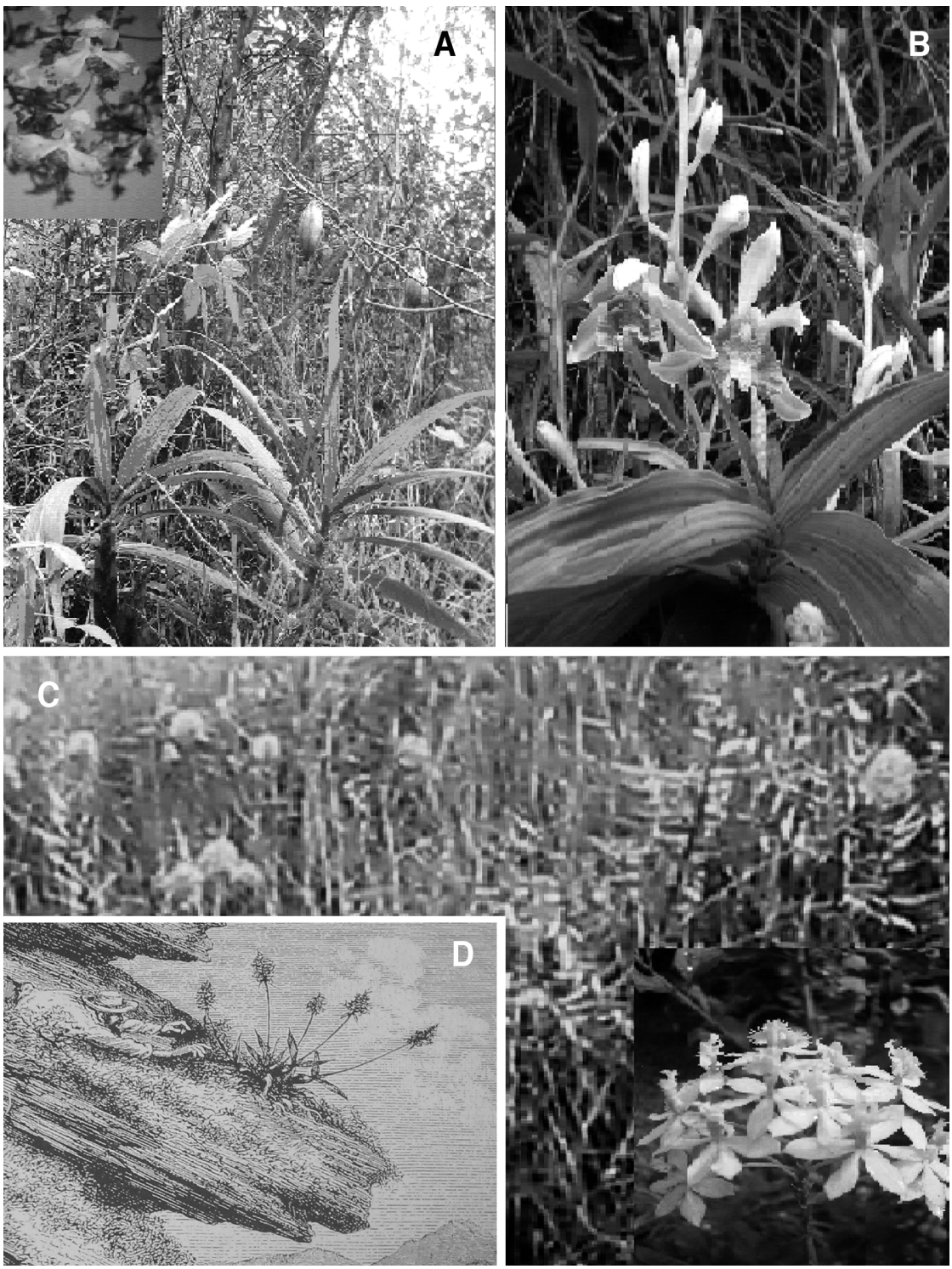


FIGURE 1. Species of open terrains and rocky outcrops. A – *Cyrtopodium punctatum*. B – *Sobralia* sp. C – *Epidendrum xanthnium*. D. *Schomburgkia* sp.

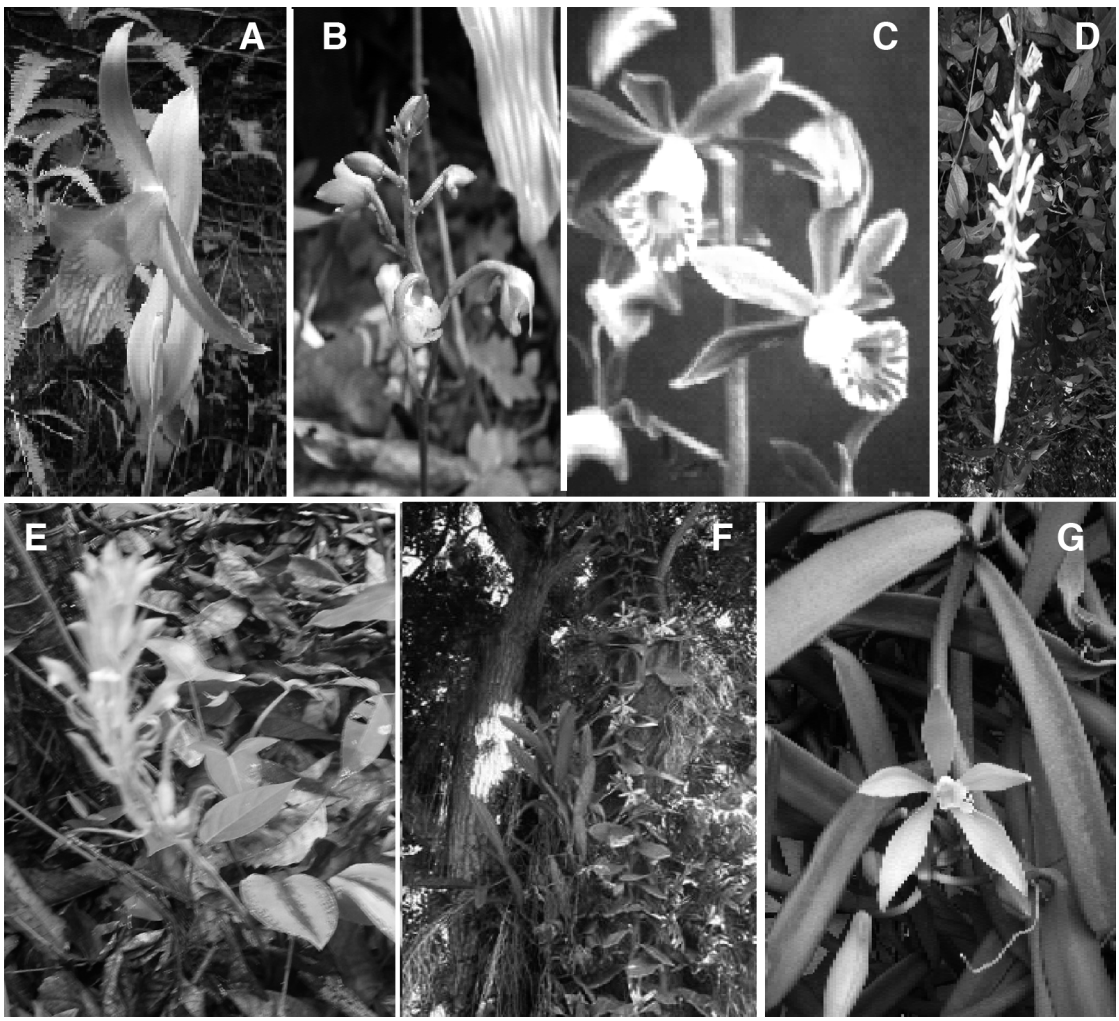
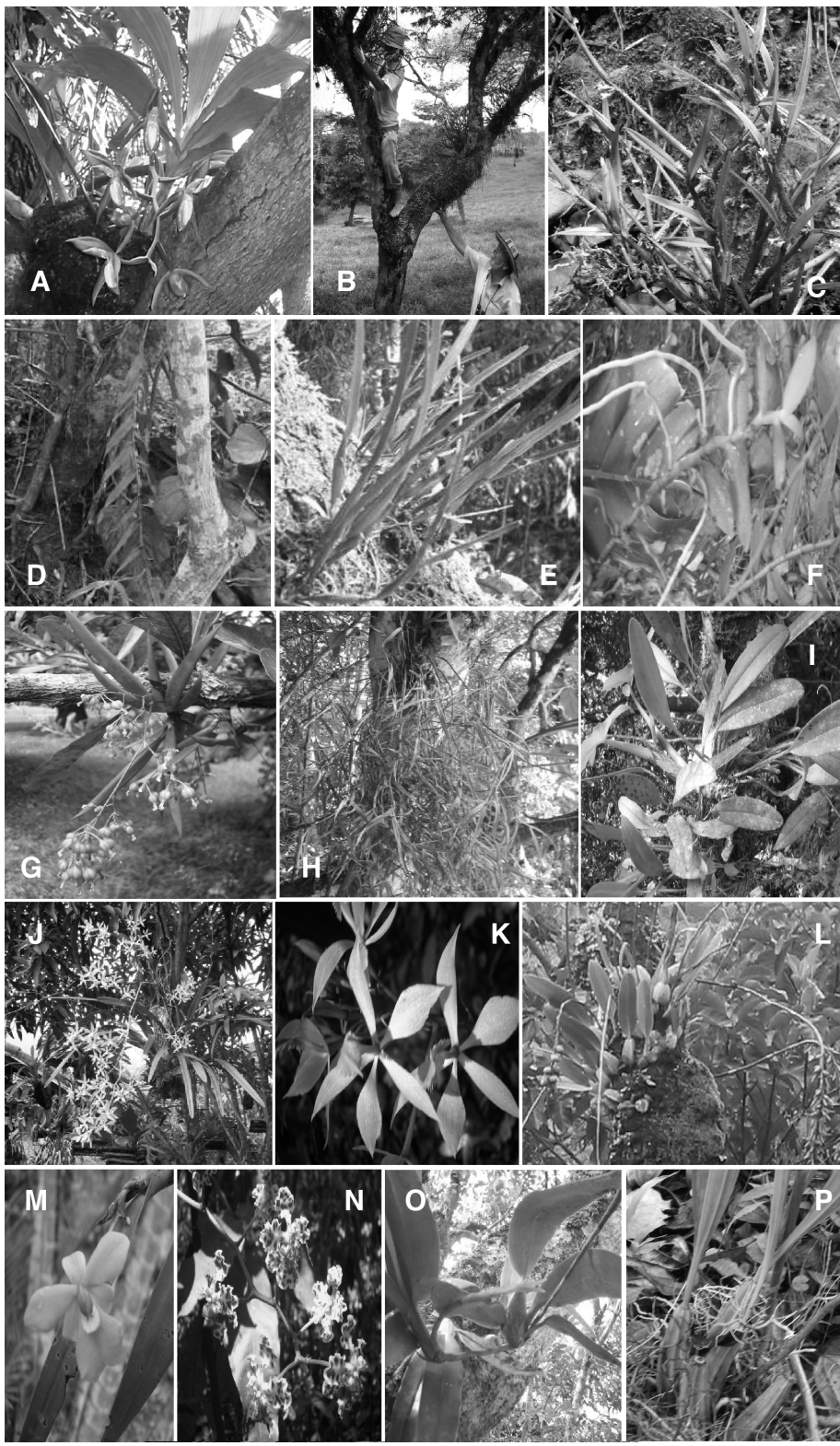


FIGURE 2. Terrestrial (A-E) and climbing (F-G) species: A – *Cleistes* sp. B – *Oeceoclades maculata*. C – *Galeandra beirichii*. D – Spiranthes sp. E – *Pelexia* sp. F – *Vanilla pompona*. G- *Vanilla odorata*.

etation during the construction of the water conduction channels to the power plants; use of round logs for construction of roads; use of hardwoods for the construction of railroad ties; use of fires to clear land for agriculture and cattle ranching; and dry season natural forest fires. Between 1910 and 1930 the regeneration process was rather slow, even though the water channel and the river provided complete protection from forest fires generated outside and above the water channels to the vegetation undergoing regeneration within the forest. The most vigorous regeneration occurred in the last fifty years, when most homes were using electricity instead of charcoal for cooking purposes. The vegetation we see today includes mature trees of 20 meters! The photographic evidence of the watershed also provides evidence that the forest of the Garden is not a recent relict but a vigorous regeneration favored by the water channels and the river which isolated two forest fragments one of 11.5 hectares (now the Botanical Garden) and a 26 hectare plot just a couple of kilometers west of the Garden. Thus, the forest cover found today in the CBG (and in various places in the basin) is the consequence of vigorous regeneration processes. A continuous secondary succession process has taken place which started in an opened field dominated by grasses with little arboreal vegetation and rather distant sources of plants for colonization more than four kilo-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meters and at least 300 meters of altitudinal difference to the nearest continuous forest patch.

THE TROPICAL DRY FOREST. The Tropical dry Forest (Bs-T) is a vegetation formation with continuous forest cover between 0-1,000 m in altitude and temperatures above 24º C and average annual rainfall between 700 and 2,000mm, with one or two dry periods per year (Espinal 1985; Murphy & Lugo 1986; Institute von Humboldt 1997). The Bs-T represents about 50% of the forested areas of Central America and 22% of South America (Murphy & Lugo 1986). In Colombia this formation is found in the Caribbean region and in the interandean valleys of the rivers Magdalena and Cauca in an area which presumably covered about 8,146,000 hectares (Espinal & Montenegro 1977). The Tropical dry Forest is one of the most threatened ecosystems of the Neotropics (Janzen 1987). In Colombia it is one of the most degraded and fragmented, with estimates of present total cover of less than 1.5% of the original cover (Etter 1993). Of this total the greatest proportion is found in the arid peri-caribbean belt with more than 6 million hectares and the NorAndean -Chocó-Magdalena province with about one million hectares (Espinal and Montenegro 1977;Hernández et al. 1992). The dry forest of the upper Cauca river valley, the main tributary of the Magdalena river, originally covered about 300,000 hectares in the Department of Valle del Cauca. Presently, the dry forest has practically disappeared to the advance of sugarcane cultivation, the major economic crop of the State. It is estimated that the cover of this formation in the Cauca Valley is less than 3,000 hectares with documented reductions of 66% between 1957 and 1986 (CVC 1994). Only a few forest relicts remain, all below 16 hectares each. The situation is only slightly less dramatic along the piedmont areas of the Central and Western Andean ranges where a few remnants and regenerated forests exist

FIGURE 3. Epiphytic species. A- *Catasetum tabulare.* B- *Dimerandra* sp. C - *Cladobium violaceum*. D – *Epidendrum* sp. E – *Epidendrum* sp. F – *Campylocentrum* sp. G. *Trizeuxis falcata*. H– *Epidendrum* sp. I – *Stelis* sp. J –*Encyclia ceratistes*. K – *Epidendrum* cf.*flexuosum*. L – *Bulbophyllum meridense.* M – *Dimerandra emeraginata*. N – *Oncidiun carthagenense*. O – *Maxillaria* sp. P. *Coryanthes* sp.

THE TROPICAL DRY FOREST OF THE MIDDLE CALI RIVER WATERSHED. The species found at the CBG and middle Cali river comprise an arrangement of secondary succession species, with a level of species richness comparable to those of other dry forests formations in the Cauca River valley (Gonzalez and Devia 1995, Orejuela 2006). The total number of 49 tree species is lower than the average number of 58.1, n= 8 sites) found by Gentry (1995). The forest of the Garden shows a notorious dominance of six tree species which in terms of numbers are ranked as follows: Arrayán (*Myrcia popayanensis*), Laurel Jigua (*Cynammomum triplinerve*), Sangregao (*Crotón gossypifolius*), Guácimo (*Guazuma ulmifolia*), Chiminango (*Pithecellobium dulce*) y Chagualo (*Clusia sp*). The vegetation of the lower stratum is heavily dominated by Cordoncillo *Piper sp* and Anamú (*Petiveria alliacea*) Phytolaccaceae family, *Croton* and individual plants of the dominant middle and upper strata. Associated to the forest there is a profusion of climbing and liana species. Among these species the Aristolochia (two species), Passiflora (four species) and Cucurbitaceae are noteworthy. The species of medium levels are: Sangregao (*Croton* two species), Arrayán (*Myrcia*, two species), Guava (*Psidum guajava*), Verraquillo (*Trema micrantha*), Carbonero (*Calliandra pittieri*), Jigua (*Cynammomum*), Guácimo (*Guazuma*), *Leucaena*, Chagualo (*Clusia*), *Solanum* and *Miconia* spp (Orejuela 2006b).

THE CBG FOREST COMPARED WITH MATURE RELICT FORESTS. In comparisons with other forests found in the Andean piedmont areas of similar size and level of connectivity with other forest fragments the CBG registers slightly lower species richness and the species composition differs in several key species. For example, in the municipality of Jamundi just south of Cali, the Ecological Reserve of Miravalle, in the Calichal river (afflue nt of the Jamundí river), and the piedmont forests along the Rio Claro (Hacienda La Novillera) the dominant species are Cascarillo (*Laderbergia magnifolia*), Tumbamaco (*Didimopanax morototoni*), Niguitos (*Miconia spp*), Balso (*Ochroma lagopus*), Ceiba (*Ceiba pentandra*), Caracolí (*Anacardium excelsum*), Algarrobo (*Hymenaea courbaril*), *Madroño (Garcinia madruno), Dinde (Maclura* tinctoria), Cañafístula (*Cassia grandis*), Cedro (*Cedrella odora-*

TABLE 1. Orchid species present and growth mode presented in the Cali river basin.

Growth mode	Species
1. Open terrain and rocky outcrop	*Cyrtopodium paniculatum*
	Sobralia
	Epidendrum xanthinum
	Schomburgkia cf. *superba*
2. Terrestrial	*Cleistes rosea*
	Galeandra beyrichii
	Oceoclades maculata
	Pelexia sp.
	Spiranthes sp. (?)
	Catasetum ochraceum
3. Climbers	*Vanilla odorata*
	Vanilla pompona
4. Epiphytic	*Catasetum tabulare*
	Dimerandra emarginata (stenopetala)
	Epidendrum spp
	Maxillaria spp
	Cladobium
	Lepanthes
	Ornithocephalus
	Stelis
	Trixeusis falcata
	Enciclia ceratistes
	Epidendrum cf. *flexuosum*
	Bulbophyllum meridense
	Oncidium carthagenense
	Campylocentrum micranthum
	Coryanthes sp .

ta), Samán (*Albizzia saman*), Catasetum *tabulare*, Orejero *(Enterolobium cyclocarpum*), Azulito (*Petrea rugosa*), Siete Cueros *(Machaerium capote),* Guáimaro (*Brosimum alicastrum*), Caimo (*Chrysophyllum argenteum)*, Guácano (*Oxandra espintana*), Cámbulo (*Erythrina glauca* and *E. poeppigiana*), Cachimbo or Pízamo (*Erythina*), Palma cuesco (*Attalea* (*Scheelea) butyraceae*), Rose and Yellow Guayacanes (*Tabebuia rosea* and *T. chrysantha*), Totocal (*Achatocarpus nigricans*). Although this zone is slightly wetter (1.300 a 1.400 mm) than the Cali river basin (900-1,000mm), the difference in species composition is notorious in the presence of mature tropical dry forest species. The relict forest of the valley floor and the piedmont areas showed a vegetation typical of late stages of the ecological succession.

Discussion

The age of continuous regeneration processes is an important factor in the species composition of a secondary forest. The early pioneering species have special competitive and reproductive abilities.

Their capacity to establish themselves in harsh conditions is remarkable. This was evidenced in the site where the Cali Botanical Garden is located today. In addition to being good dispersers and colonizers, they are tolerant to difficult climatic and edaphic conditions like solar exposure, scarcity of nutrients, compacted soils. Many species are also tolerant of forest fires or they are opportunistic to take advantage of the bursts of nutrients following the fire events. Additionally, it is the experience of the authors that these species recuperate rapidly after the foraging voracity of Harvester Ants (*Atta cephalotes*). This relative tolerance or resistance confers them short and medium term advantages over competing plant species. When the species of plants establish themselves in the plot, they benefit directly from the soil improvement the ants bring to the sites. It is noteworthy that the six dominant species in the Garden are also among the species most readily consumed by the ants!

Should there be more orchid species in the Cali river watershed? In the absence of previous inventories of species one would have to say that since the forest was completely cleared late in the IXX century and early in the XX the number of orchid species would have depended on the kind of regeneration process which took place since that period. After the major disturbance of the forest to establish the water conduction channel for the hydroelectric power plant, the cleared area was left alone. The initial forest received the benefit of passive protection year after year, during a period that is evident today. The forested area enclosed between the Cali river and the water channel formed a solid fire break and the forest regeneration process advanced unchecked. The result is a regenerated forest with trees which reach 20m The fact that the forests of this part of the watershed are loosely interconnected with premontane (subtropical) and lower montane forests provide a biological corridor where many plant and animal species move. The possibilities for establishment of orchids is favored by the wind currents which move up and down the corridor on a daily basis with seasons was moderate to strong winds. Therefore, there has been opportunities for species enhancement during nearly one century.

A likely answer to the question could be that there are relatively few orchid species present in the watershed. Without doubt this is true for the regenerated forest compared with a similar sized relictual forest. Such relictual forest still exist in the Rio Claro, Jamundí and Pance rivers. In these three forests, the number of species is considerably higher than in the Cali river at any given altitudinal range. However, if one considers forest regenerations of the same age, it is almost sure that the protected forest of the Cali river corridor would have not only more tree species but also many more orchid species. With all certainty the presence of a diversified vegetation in different growth forms (canopy trees, understory trees and shrubs, ground vegetation, lianas and climbers and epiphytes and hemiepiphytes) would have the potential to host a greater richness of orchids as well. The higher humidity and the greater amount of shade favored by mature forests also favors the presence of orchids, particularly the epiphytic kinds. The effect of the prolongued deforestation, with a relatively long period before the succesional process could gain momentum, slowed down the orchid species packing process. This early period of the secondary succession was also characterized by a temporary loss of orchid pollinator species.

Conclusions and recommendations

It is clear that under suitable protective conditions even a highly degraded forest will develop toward a reasonably diversified state. Along with the forest regeneration process, the orchid species will also profit, both in terms of the species numbers and density of individual populations. But additionally, should there be a variety of ecosystem types in the watershed characterized by forests of various stages of maturity, gallery and riparian vegetation, presence of microwatershed systems with opened areas, with grasslands and rocky outcrops, one would have a situation which would favour a solid accumulation of species. From this consideration, the following recommendations are offered:

- To use the identified pioneer species of the tropical dry forest as ideal germplasm of native species to promote vegetation enrichment and restorative

processes in degraded interandean valley floors and hillsides. Only in the Cauca River valley these areas cover in excess of 200,000 hectares.

- To enrich the forest of the Cali Botanical garden and surrounding areas along the biological corridor of the Cali river with species (including the orchids) found in nearby relicts of mature tropical dry forest. These enrichments would in a sense mimic advanced stages of secondary regeneration. Nursery trials with these species would be of paramount importance.
- To promote the conservation of regenerated forests in all altitudinal levels in the interandean river valleys, particularly where the vegetation cover has been most severely affected by human activities, like in the piedmont areas (1.000-1.300m, coffee belt region (1,300-1,700m) and in the sugar cane zone (1,000m). The connexion of these two areas through biological corridors would generate great environmental and socio-economic benefits.
- To favor forest regeneration processes where extensive cattle ranching is presently being conducted. There are important sustainable silvopastoral alternatives available which would intensify the cattle production with significant reductions in the area devoted to pastures.
- To use the native orchids of the tropical dry forest as key elements of an environmental interpretation program in the Cali Botanical Garden. Similar orchid gardens could be established as school projects in the city.
- To develop and maintain an intensive effort to reduce forest fires in the watershed.
- To prevent the excessive clearing of road banks which frequently become festooned with orchids species.

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LITERATURE CITED

CVC. 1994. Informe 90-7. Comparación de la cobertura de bosques y Humedales entre 1957 y 1986 con delimitación de las comunidades naturales críticas del valle geográfico del Río Cauca. Cali, Documento interno. CVC.

Espinal, L.S. 1985. Geografía ecológica del departamento de Antioquia Revista de la Facultad Nacional de Agronomía, 38 (1): 24-39.

Espinal, L.S. & E. Montenegro. 1977**.** Formaciones vegetales de Colombia. Instituto Geográfico Agustín Codazzi, Bogotá, pp 201 .

Etter, A. 1993. Diversidad ecosistémica en Colombia hoy. Pp 43-61 in Nuestra diversidad biótica. CEREC & Fundación Alejandro Angel Escobar.

Gentry, A.H. 1995. Diversity and floristic composition of neotropical dry forest. Pp. 116-194. in: Tropical deciduous Forest Ecosystem. S. Bullock, E. Medina & H.A. Mooney (eds). Cambridge Univ. Press, Cambridge.

González, S. & W. Devia. 1995. Caracterización fisionómica de la flora de un bosque seco secundario en el corregimiento de Mateguadua, Tuluá, Valle. Cespedesia Vol 20 No 66: 35-63.

Hernandez, C., T. Walschburger, R. Ortiz & A. Hurtado, 1992. Sobre origen y distribución de la biota suramericana y colombiana. Pp. 55-104 in: Diversidad biológica en Iberoamerica, G. Halffter (compiler). Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo, Instituto de Ecología, Secretaría de Desarrollo, Mexico, D.F., Mexico.

Instituto Alexander von Humboldt (Programa de Inventario de la Biodiversidad, Grupo de Exploraciones y Monitoreo Ambiental GEMA). 1997. El Bosque seco Tropical (Bs-T) en Colombia.

Janzen, D.H. 1987. Insect diversity of a Costa Rican dry forest: Why keep it. Biol. Journal Linn. Soc. 30: 343-356.

Murphy, P.G. & A.E. Lugo. 1986. Ecology of tropical dry forest. Ann. Rev. Ecol. Syst. 17 : 67-68 .

Orejuela, J. 2005. An integrated approach to orchid conservation in Colombia: what do orchids, hummingbirds, bears, potable water, and indigenous land rights have in common? 2nd International Orchid Conservation Congress, Sarasota, Florida, USA. Selbyana 26 1-(2):32-45.

Orejuela, J.E. 2006. The Cali Botanical Garden and the Conservation of Ecosystems in the Cali River basin, Cali, Colombia. Lyonia March, 2006. WWW.lyonia.org/view Article.php?articleID=472.

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HOTSPOTS OF NARROW ENDEMISM: ADEQUATE FOCAL POINTS FOR CONSERVATION IN *DENDROCHILUM* (ORCHIDACEAE)

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KEY WORDS: area-selection, biodiversity, biogeography, complementarity, Malesia, orchids

The general aim of conservation is to ensure persistence of biodiversity value. Given certain measures (financial, logistic, etc.) the specific goal must be to maximize the amount of biodiversity value to be secured by these means. Several area selection methods are available for such purpose, and they represent very different conservation philosophies (Williams *et al.* 1996; Humphries 2006). Two fundamentally different approaches exist: (1) locating hotspots of species richness or narrow endemism, and (2) designating conservation areas according to complementarity methods.

Some authors define hotspots as areas with exceptional species richness or concentrations of endemic species and experiencing exceptional loss of habitat (e.g. Myers 1988; Myers *et al.* 2000). Frequently, however, the last criterion is disregarded (e.g. Prendergast *et al.* 1993; Williams *et al.* 1996) – a practice that I have chosen to adopt in the present study. Selecting hotspots of (species) richness has been a popular method, and with appropriate qualification hotspots can be used for high-scoring areas on any value scale and on any spatial scale (Humphries 2006). One advantage of the hotspot approach is that it deals with species occurrence data with apparent quantitative rigour. Hotspots of narrow endemism resemble hotspots of richness, but only endemic taxa are taken into account. As noted by Humphries (2006) this has the advantage of requiring data for only a subset of the species, and it is more likely to select for more highly complementary areas.

Complementarity methods are applied to designate the smallest selection of areas that in combination represent the maximum level of diversity (without necessarily including any hotspots). Complementary areas are generally more efficient than hotspots of either richness or rarity (Humphries 2006). The drawback of complementarity methods is that they either demand exhaustive searches using linear programming algorithms, or depend on heuristic algorithms that may not find optimal solutions (Csuti *et al.* 1997).

Taxonomic diversity (usually at species level) is by far the most commonly used measure of biodiversity. However, taxic diversity (Vane-Wright *et al.* 1991) and phylogentic diversity (Faith 1992; Mace *et al.* 2003; Pillon *et al.* 2006) are interesting alternatives. These measures are hardly sensitive to taxonomic inflation, and they add another dimension to the evaluation of conservation priorities.

The orchid genus *Dendrochilum* Blume has an Indo-Malesian distribution, ranging from Myanmar in the northwest across peninsular Thailand, Malaysia, Indonesia and the Philippines to southernmost Taiwan in the north and to Papua New Guinea in the southeast. The far majority of species are restricted to cool, humid, and often exposed conditions of montane forests. The genus contains an unusually high share of narrow endemics, and pronounced centres of species diversity are found in the high mountains of the Philippines (Pedersen 1997a), Borneo (Wood 2001), and Sumatra (Comber 2001). Surprisingly, only one *Dendrochilum* species is known from the mountain-rich island of New Guinea. The latest global taxonomic survey of *Dendrochilum* was that of Pedersen *et al.* (1997). Based on this checklist and subsequent changes, 268 species are currently accepted (Pedersen, unpubl. data).

No species of *Dendrochilum* are included in IUCN's latest red data list based on the global conservation status of individual species (http://www.redlist.org). However, among the 18 *Dendrochilum* taxa considered endemic to Sarawak in Borneo, Beaman *et*

al. (2001) classified four species as vulnerable (VU), seven species and one variety as endangered (EN), and four species and one variety as critically endangered (CR). Only *D. globigerum* (Ridl.) J.J.Sm. was not regarded as threatened with extinction in the wild! Taking into account historical and current deforestation rates throughout most of Malesia and their estimated impact on orchid populations (Koopowitz 2001; Koopowitz *et al.* 2003) there is every reason to believe that corresponding analyses elsewhere would give a similarly gloomy result.

Evidently, active measures are urgently needed to protect representative taxonomic and phylogenetic diversity in *Dendrochilum*. Due to the unusually high share of narrow endemics in the genus, it is tempting to focus on hotspots of narrow endemism when setting the geographic conservation priorities. In the present study the conservational adequacy of focal points selected as hotspots of narrow endemism will be assessed by parallel evaluation of complementarity and of the overall level of diversity covered by this method.

Methods

The study was based on 22 semi-natural range units (Table 1). Among the 268 accepted species of *Dendrochilum*, the following had to be excluded from the analysis due to insufficient, unconfirmed, or entirely lacking distribution data: *D. barbifrons* (Kraenzl.) Pfitzer, *D. coccineum* H.A. Pedersen & Gravend., *D. croceum* H.A. Pedersen, *D. exalatum* J.J.Sm., *D. panduratum* Schltr., *D. warrenii* H.A. Pedersen & Gravend. For all species included, distribution data were extracted from the following sources: Smith (1933), Pedersen (1997a, 1997b, 2001), Pedersen *et al.* (1997, 2004), Beaman *et al.* (2001), Comber (2001), Wood (2001). Records explicitly based on uncertain identifications were disregarded. Infrageneric taxa above species level were designated according to Pedersen *et al.* (1997).

All range units were sorted in ascending order by their individual numbers of endemics, and the cumulative percent of endemics was plotted against that of the range units to form a Lorenz curve (Weiner & Solbrig 1984; Calvo 1990). Hotspots of narrow endemism were then designated as the range units defining the steep part (dy>dx) of the curve.

TABLE 1. Survey and definitions of the 22 range units that were applied in the analyses of overall diversity patterns of *Dendrochilum*.

1	Myanmar
2	Thailand
3	Taiwan
4	N Philippines (Babuyan Islands, Batan Islands, Catanduanes, Luzon, Marinduque, Mindoro, Polillo Islands)
5	W Philippines (Balabac, Calamian Group, Palawan)
6	S Philippines (Basilan, Camiguin, Dinagat, Mindanao, Siargao, Sulu Archipelago)
7	C Philippines (remaining Philippine Islands)
8	N Borneo (Sabah)
9	NW Borneo (Brunei, Sarawak)
10	W Borneo (Bunguran, Kalimantan Barat)
11	S Borneo (Kalimantan Selatan, Kalimantan Tengah)
12	E Borneo (Kalimantan Timur)
13	Peninsular Malaysia/Singapore
14	N Sumatra (Aceh, Sumatera Utara)
15	E Sumatra (Bangka, Jambi, Lampung, Riau, Sumatera Selatan)
16	SW Sumatra (Bengkulu, Sumatera Barat)
17	Java
18	Lesser Sunda Islands
19	Sulawesi
20	Maluku
21	Irian Jaya
22	Papua New Guinea

To compare regional exploration histories and evaluate the reliability of current interpretations of distribution patterns in *Dendrochilum*, a cumulative graph of narrow endemics as function of time was prepared for each hotspot. A cumulative graph of non-endemics in the entire geographic range was included for comparison.

Obviously, designation of hotspots of narrow endemism automatically secures a high degree of complementarity among the areas selected as focal points for conservation in a genus dominated by narrow endemics. However, this method does not necessarily ensure significant complementarity with regard to the representation of non-endemic species. To evaluate this problem, cluster analysis and ordination of all range units were performed on data for non-endemics only. Prior to the analyses, each non-endemic species was scored as present (1) or absent (0) in each range unit. All statistic operations were performed using the program NTSYSpc 2.0 (Rohlf 1998).

In the cluster analysis, floristic similarity was calculated for each pair of range units by the DICE algo-

rithm (Dice 1945). The resulting distance matrix was used to construct a dendrogram describing the floristic similarity among all range units. The dendrogram was constructed by means of the UPGMA (unweighted pair-group method using arithmetic averages) algorithm (Legendre & Legendre 1983). UPGMA is a polythetic agglomerative technique that appears to maximize the cophenetic correlation, and its use is recommended when there is no specific reason for choosing some other clustering technique (Sneath & Sokal 1973).

Ordination was performed as principal components analysis (PCA; Sneath & Sokal 1973). PCA is suited for the first iteration of analyses, because each character is given the same a priori weight, whereas intergroup distances are not taken into account. This method was originally developed for quantitative characters, but can also be used on binary characters (Gower 1966; Dunn & Everitt 1982).

The extent to which hotspots of narrow endemism are identical with areas representing high levels of overall taxonomic diversity was assessed by direct comparison facilitated by parallel ranking of range units according to their relative individual richness at species, section, and subgenus level, respectively. For each taxonomic level the maximum taxon score was set at 100%, and the lower scores were converted to percentages accordingly. In this way, relative taxonomic richness could be compared directly across taxonomic levels.

To obtain an estimate of the extent to which hotspots of narrow endemism ensure complementarity at higher taxonomic level, geographic affinities of regional *Dendrochilum* floras, characterized by diversity at section level, were summarized by ordination (PCA, see above). Two analyses were performed – one in which each section (or subgenus, if not subdivided further) was scored as present (1) or absent (0) in each range unit; and one based on the number of species representing each section in each range unit. In the latter analysis, all characters (sections) were standardized prior to analysis (Gower 1971).

Results

The relative distribution of narrow endemics among range units can be seen from the Lorenz curve (Fig. 1). Seven range units define the steep part

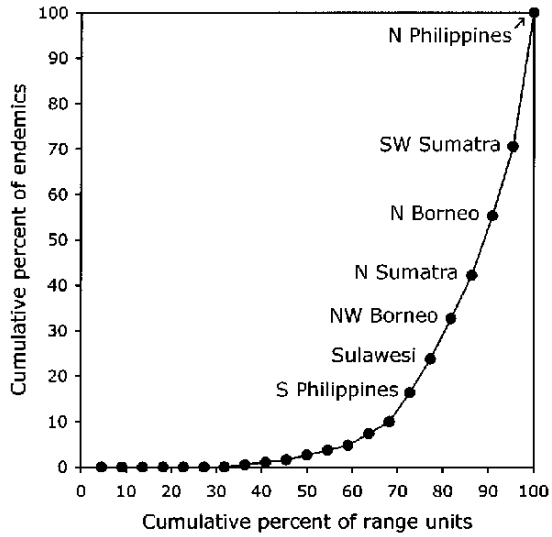

FIGURE 1. Lorenz curve demonstrating the markedly heterogenous distribution of narrowly endemic *Dendrochilum* species among the 22 range units. Seven range units make up the steep part of the curve (dy>dx) and are designated as hotspots of narrow endemism.

(dy>dx) of the curve and can accordingly be designated as hotspots of narrow endemism: N Philippines (56 endemics), SW Sumatra (29), N Borneo (25), N Sumatra (18), NW Borneo (17), Sulawesi (14), S Philippines (12).

The regional exploration histories of the seven hotspots of narrow endemism are illustrated in Fig. 2. Two distinct periods of exploration exist, that is approximately 1900–1940 and 1985–2000. This pattern is clearly reflected also by the general graph for non-endemic species, although a higher share of the non-endemics were described prior to 1900.

According to both the cluster analysis (Fig. 3) and the PCA (Fig. 4), performed on data for non-endemic species only, four groups of hotspots of narrow endemism are highly complementary: N/SW Sumatra, Sulawesi, N/NW Borneo, N/S Philippines. In the PCA (Fig. 4), Sulawesi groups together with all remaining range units except C Philippines, but this is not obvious from the cluster analysis (Fig. 3). Indeed, the cluster analysis suggests higher complementarity and more pronounced geographic grouping among the non-hotspots than is evident from the PCA (Fig. 4).

In Table 2 all range units are ranked according to relative taxonomic richness at species, section and

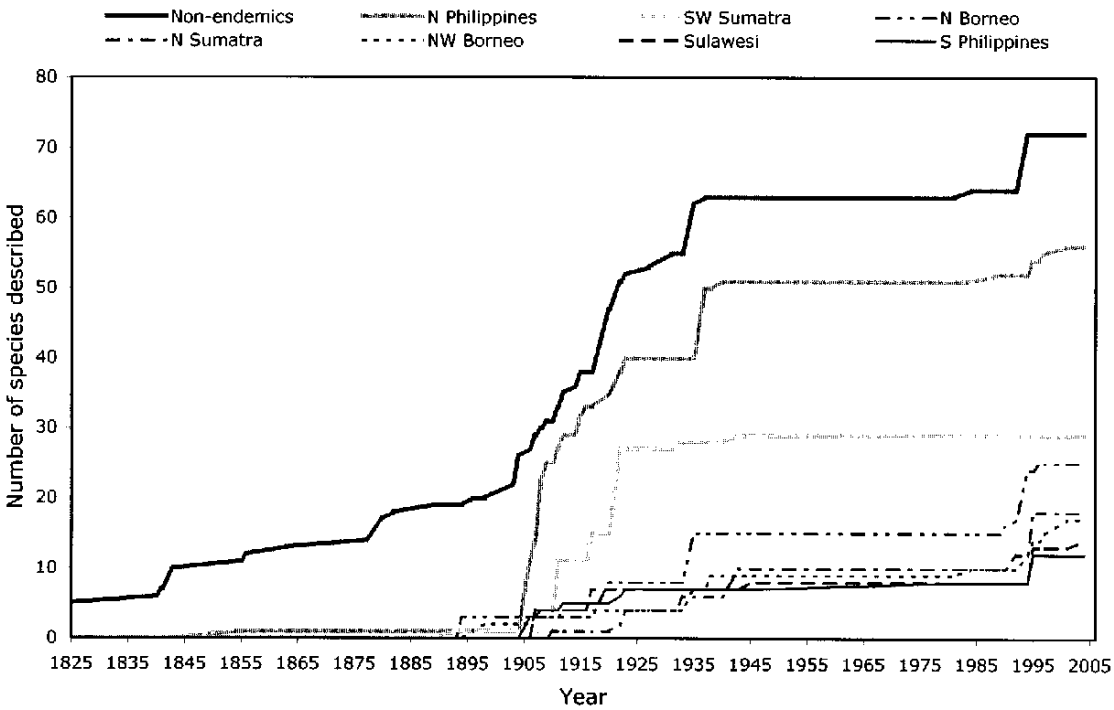

FIGURE 2. Cumulative graphs illustrating the exploration histories of regional endemic *Dendrochilum* floras. For all recognized hotspots of narrow endemism, a cumulative graph of endemics described from 1825 to 2005 is given. A cumulative graph based on all non-endemics is included for comparison.

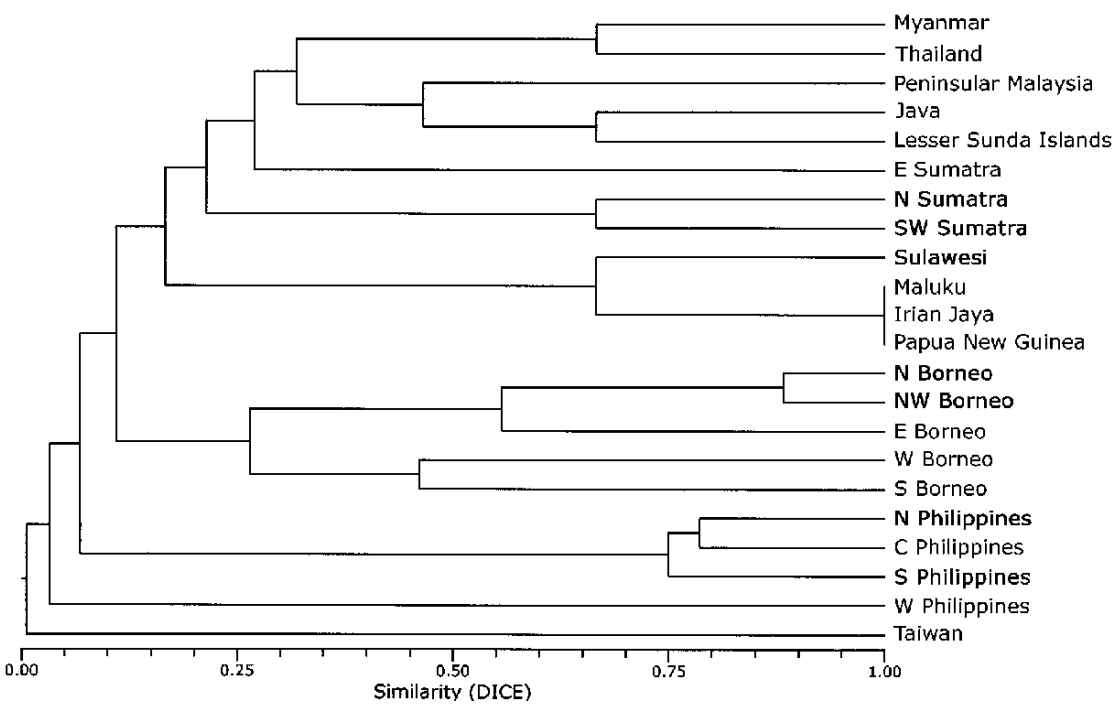

FIGURE 3. Dendrogram showing the similarities of regional *Dendrochilum* floras (non-endemic species only).

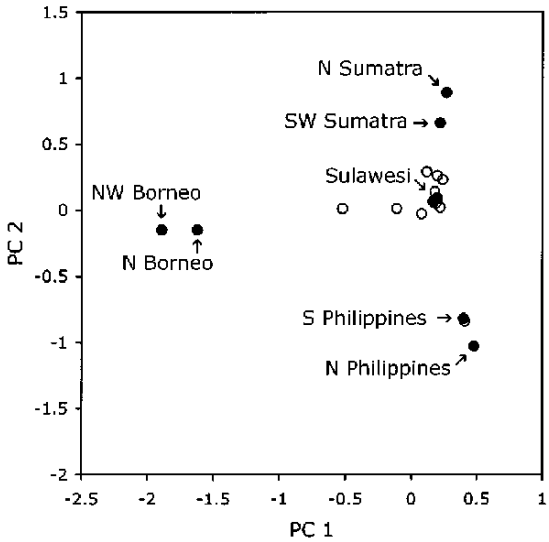

FIGURE 4. Mutual affinities of regional *Dendrochilum* floras. Plot from the first two principal components from the PCA performed on distribution data for non-endemic species only. Filled symbols represent hotspots of narrow endemism. The variation was 36.1% along PC axis 1 and 18.9% along PC axis 2.

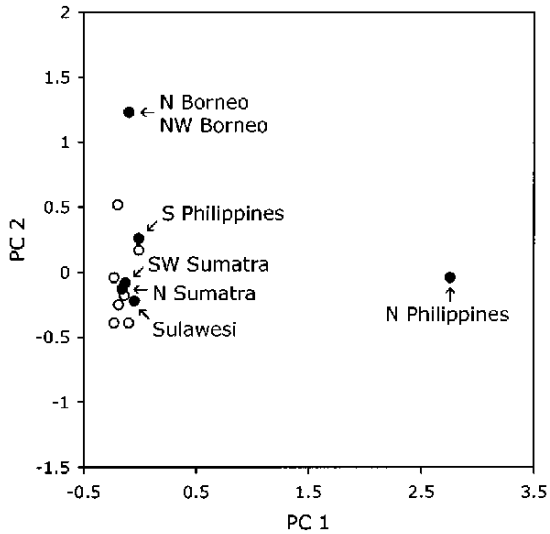

FIGURE 5. Mutual affinities of regional *Dendrochilum* floras. Plot from the first two principal components from the PCA performed on presence/absence data for sections. Filled symbols represent hotspots of narrow endemism. The variation was 38.5% along PC axis 1 and 21.1% along PC axis 2.

subgenus level, respectively, and in each column the hotspots of narrow endemism are highlighted to facilitate comparison.

According to both PCAs performed using sections as characters, N Philippines and N/NW Borneo appear highly complementary to each other and to all remaining range units (Figs 5–6). Within the latter group, the two analyses gave diverging results. Thus, according to the analysis based on presence/absence data (Fig. 5) Sulawesi groups together with N and SW Sumatra. In the plot from the analysis based on frequency data, on the other hand, Sulawesi is situated much closer to S Philippines (Fig. 6).

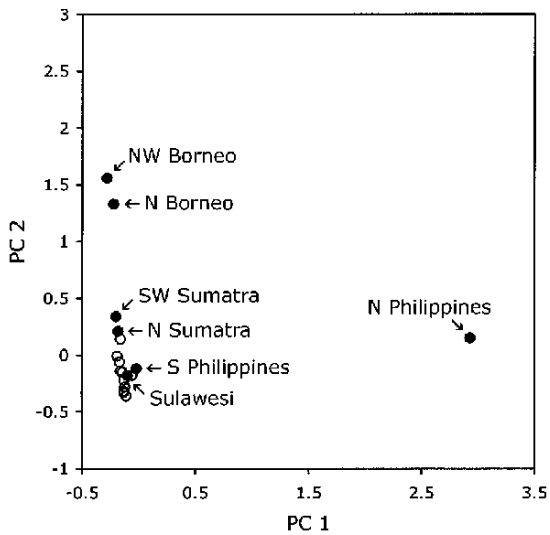

FIGURE 6. Mutual affinities of regional *Dendrochilum* floras. Plot from the first two principal components from the PCA performed on frequency data for sections. Filled symbols represent hotspots of narrow endemism. The variation was 43.2% along PC axis 1 and 26.1% along PC axis 2.

Discussion

NARROWLY ENDEMIC SPECIES. It appears directly from the Lorenz curve (Fig. 1) that the seven hotspots, though constituting less than 30% of the range units, hold nearly 90% of the narrow endemics. Consequently, using hotspots of narrow endemism as focal points for conservation is a very qualified method for securing a high share of this species group in *Dendrochilum*. Furthermore, it should be remembered that narrowly endemic species make up 71% of the genus.

Obviously, the credibility of the above finding depends on the reliability of current interpretation of geographic diversity patterns. After all, the steady rate at which new orchid species have been described over the past 25 years seems to continue (Cribb & Govaerts 2005), and this might continuously change apparent geographic diversity patterns in several genera, including *Dendrochilum*. However, notwithstanding the heterogenous rate of exploration of each hotspot recognized in the present study, the hotspots constituting top-four (and their relative mutual importance) have remained unchanged for more than 60 years (Fig. 2). It should be noted that the graphs do not reflect historical perceptions of diversity patterns. They simply summarize the exploration histories of regional *Dendrochilum* floras according to current taxonomic and geographic interpretation. The observed constancy in diversity patterns over time indicates that current designation and ranking of most-important hotspots of narrow endemism can be considered sufficiently reliable.

Non-endemic species. – Three important observations can be made from Table 2: (1) the six range units with highest general species richness are identical with six hotspots of narrow endemism; (2) N Philippines, N and NW Borneo constitute top-three at both species and section level, and (3) the maximum subgenus score is shared among N and NW Borneo, N and S Philippines (and the non-hotspot C Philippines). Generally speaking, the most prominent hotspots of local endemism largely coincide with the range units showing the greatest taxonomic diversity in general and the greatest species richness in particular.

In order to maximize the level of complementarity (considering non-endemic species only), the areas of highest conservation priority should be selected among (rather than within) the four groups of hotspots that can be recognized in Figs 3–4 (viz. N/S Philippines, N/NW Borneo, N/SW Sumatra, and Sulawesi). With proper consideration, the use of hotspots of narrow endemism as focal points for conservation will also ensure a high level of complementarity with regard to non-endemic species.

Taxonomic versus phylogenetic diversity. – In the Philippine *Dendrochilum* flora, a clear correlation exists between the degrees of endemism and restrictedness to higher altitudes. If compared with distribution patterns expected for species having evolved before or after the Pleistocene, respectively, this correlation suggests that the majority of living (sub)montane species of *Dendrochilum* (including the far majority of narrow endemics in the Philippines) have evolved after the Pleistocene (Pedersen 1997a). Based on corresponding distribution data, Wood (2001) proposed a similar scenario for Borneo. The hypothesis that narrow endemics in *Dendrochilum* are largely (or universally) neoendemics resulting from local evolutionary radiation at high altitudes is consistent with preliminary molecular data (Barkman & Simpson 2001).

The evolutionary hypothesis outlined above accentuates the importance of hotspots of narrow endemism in *Dendrochilum*, as conservation of "cradles of diversity" is now often considered a priority (Mace *et al.* 2003). At the same time, however, the hypothesis implies that using hotspots of narrow endemism as focal points for conservation in *Dendrochilum*, though ensuring a high species diversity, does not necessarily ensure a high phylogenetic diversity. In principle, the high species richness encountered in each hotspot of narrow endemism might represent recent prolific radiation at the end of just one major lineage – and not all such lineages might be secured if conservation efforts are directed to a few selected areas only. A marked discrepancy between geographic patterns of species diversity and estimated phylogenetic diversity, though on a different background, was recently demonstrated in the orchid genus *Dactylorhiza* (Pillon *et al.* 2006), and this potential complication for setting geographic conservation priorities should be considered for *Dendrochilum* as well.

No major cladistic analysis of *Dendrochilum* is yet available, so the geographic patterns of phylogenetic diversity cannot be estimated properly. However, the latest infrageneric classification of *Dendrochilum* (Pedersen *et al.* 1997), though not based on cladistic analysis, was hypothesized by the authors to reflect overall phylogenetic relationships in the genus. Tentatively accepting this hypothesis, geographic patterns of taxonomic diversity above species level can

TABLE 2. Geographic diversity in *Dendrochilum* – parallel ranking of range units according to their relative individual richness at species, section, and subgenus level, respectively. For each taxonomic level the maximum score has been set at 100% and the lower scores converted accordingly. Hotspots of narrow endemism are given in bold. Range units with identical scores are listed alphabetically in each column. 100% scores correspond to 71 species, 8 sections, and 3 subgenera, respectively.

SPECIES		SECTIONS		SUBGENERA	
N Philippines	100	**N Philippines**	100	C Philippines	100
N Borneo	79	**N Borneo**	86	**N Borneo**	100
NW Borneo	76	**NW Borneo**	86	**N Philippines**	100
SW Sumatra	59	E Borneo	57	**NW Borneo**	100
N Sumatra	49	**S Philippines**	57	**S Philippines**	100
S Philippines	36	C Philippines	43	E Borneo	50
E Borneo	24	**N Sumatra**	43	E Sumatra	50
Sulawesi	21	E Sumatra	29	Java	50
C Philippines	20	Java	29	Lesser Sunda Islands	50
Java	17	Lesser Sunda Islands	29	Myanmar	50
E Sumatra	14	Peninsular Malyasia	29	Peninsular Malaysia	50
Peninsular Malaysia	14	**SW Sumatra**	29	**N Sumatra**	50
W Borneo	11	W Borneo	29	S Borneo	50
Lesser Sunda Islands	6	Myanmar	14	**SW Sumatra**	50
S Borneo	6	S Borneo	14	W Borneo	50
Myanmar	1	**Sulawesi**	14	Irian Jaya	0
W Philippines	1	W Philippines	14	Maluku	0
Irian Jaya	0	Irian Jaya	0	Papua New Guinea	0
Maluku	0	Maluku	0	**Sulawesi**	0
Papua New Guinea	0	Papua New Guinea	0	Taiwan	0
Taiwan	0	Taiwan	0	Thailand	0
Thailand	0	Thailand	0	W Philippines	0

be used as rough indirect indicators of phylogenetic diversity patterns in the genus.

It appears from Table 2 that N Philippines, N and NW Borneo constitute top-three at both species and section level, and that the maximum subgenus score is shared among N and NW Borneo, N and S Philippines (and the non-hotspot C Philippines). Consequently, the most prominent hotspots of narrow endemism at species level largely coincide with the range units showing the greatest relative taxonomic diversity at both species, section, and subgenus level. The immediate impression from Table 2 might be a marked negative correlation between this tendency and the taxonomic level, but it should be noticed that scores are less differentiated at section level, and even less so at subgenus level where only three different scores exist (Table 2).

In order to maximize the level of complementarity at sectional level, the areas of highest conservation priority should be selected among (rather than within) the three groups of hotspots that can be recognized in Figs 5–6 (viz. N Philippines, N/NW Borneo, and a group containing the remaining hotspots). With proper consideration, the use of hotspots of narrow endemism as focal points for conservation will also secure a high level of complementarity with regard to sections. The discrepancy between patterns obtained by analyses performed on presence/absence data (Fig. 5) and frequency data (Fig. 6), respectively, are small and should hardly affect selection of top-priority focal points when criteria concerning narrowly endemic species and non-endemic species are also taken into account (see above).

Does the hierarchical infrageneric classification of Pedersen *et al.* (1997) really reflect phylogenetic relationships in *Dendrochilum*? This question is obvi-

ously of critical importance. Recent cladistic analyses based on ITS sequence data have questioned the phylogenetic consistency of our generic subdivision (Barkman 2001; Barkman & Simpson 2001). However, the molecular phylogenetic analyses performed on *Dendrochilum* so far (Barkman 2001; Barkman & Simpson 2001, 2002; Pedersen *et al.* 2004) cover only a minor part of the geographic range and proposed infrageneric taxa in the genus. At present, it is evident that our infrageneric classification (Pedersen *et al.* 1997) is not completely consistent with phylogentic relationships in *Dendrochilum*, but the magnitude of inconsistency remains to be settled.

CONCLUSIONS AND PERSPECTIVES. Current interpretation of diversity patterns in *Dendrochilum* appears reliable, and the most important aspects of diversity in this genus can be adequately preserved by conservation efforts focused on hotspots of narrow endemism. Indeed, the top-three hotspots of narrow endemism (N Philippines, SW Sumatra, N Borneo) also provide near-maximum levels of complementarity (assessed for sections and non-endemic species) as well as high taxonomic richness at both species, section, and subgenus level.

Based on world-wide distribution data for vascular plants, mammals, birds, reptiles, and amphibians, combined with regional degrees of threat through habitat loss, Myers *et al.* (2000) recognized 25 "biodiversity hotspots" – defined as areas where exceptional concentrations of endemic species are undergoing exceptional loss of habitat. The 25 biodiversity hotspots contain the remaining habitats of 44% of all vascular plant species, but their cover of primary vegetation has been reduced by 88% and now constitutes only 1.4% of the Earth's land surface. According to Myers *et al.* (2000) the 25 biodiversity hotspots exhibit a 68% overlap with Birdlife International's Endemic Bird Areas, 82% with IUCN/WWF International's Centres of Plant Diversity and Endemism, and 92% with the most critical and endangered eco-regions of WWF/US's Global 200 List.

Among the biodiversity hotspots recognized by Myers *et al.* (2000), Sundaland, the Philippines, and Wallacea in combination accommodate all known species of *Dendrochilum*, and only *D. longifolium* Rchb.f., *D. pallidiflavens* Blume, and *D. uncatum* Rchb.f. extend to neighbouring regions. Thus, all important diversity in *Dendrochilum* is confined to areas that are undergoing exceptional loss of natural habitats, but also to areas of the highest conservation priority in general. However, *Dendrochilum* is not equally represented throughout the above biodiversity hotspots. On the contrary, distinct hotspots of narrow endemism are found on a regional scale within both Wallacea (Sulawesi), the Philippines (N and S Philippines), and Wallacea (N and NW Borneo, N and SW Sumatra).

The example of *Dendrochilum* highlights the need to assess biodiversity patterns on various geographic scales. Indeed, also assessments on a subregional scale would be needed to pinpoint exact conservation needs in *Dendrochilum*. Distinct local concentrations of species within the hotspots of narrow endemism recognized in the present study have been clearly demonstrated for N and S Philippines (Pedersen 1997a), as well as for N and NW Borneo (Wood 2001). Some of these small areas can even be characterized as centres of local endemism. Obviously, ample knowledge of such spatial substructures of diversity should be procured, preferably for a broad selection of organisms, and utilized in the process of area selection for conservation.

The analyses in the present study have not taken into account possible discrepancies between current and historical species occurrences, and they tell nothing about the present state of habitat fragmentation or other ecological conditions of potential conservation areas. Obviously, historical and ecological factors should be integrated in the area selection process in order to optimize the actual conservation effect (Tilman *et al.* 1994; Crisci *et al.* 2006).

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LITERATURE CITED

Barkman, T.J. 2001. Evolution of vegetative morphology in Mount Kinabalu high-elevation endemics: insights from the orchid genus *Dendrochilum*. Sabah Parks Nature J. 4: 9–24.

Barkman, T.J. & B.B Simpson. 2001. Origin of high-elevation *Dendrochilum* species (Orchidaceae) ende-

mic to Mount Kinabalu, Sabah, Malaysia. Syst. Bot. 26: 658–659.

Barkman, T.J. & B.B. Simpson. 2002. Hybrid origin and parentage of *Dendrochilum acuiferum* (Orchidaceae) inferred in a phylogenetic context using nuclear and plastid DNA sequence data. Syst. Bot. 27: 209–220.

Beaman, T.E., J.J. Wood, R.S. Beaman & J.H. Beaman. 2001. Orchids of Sarawak. Natural History Publications (Borneo), Kota Kinabalu & Royal Botanic Gardens, Kew.

Calvo, R.N. 1990. Inflorescence size and fruit distribution among individuals in three orchid species. Amer. J. Bot. 77: 1378–1381.

Comber, J.B. 2001. Orchids of Sumatra. Royal Botanic Gardens, Kew.

Cribb, P. & R. Govaerts. 2005. Just how many orchids are there? *In*: A. Raynal-Roques & A. Roguenant (eds), Actes du 18e congrès mondial et exposition d'orchidées, 11-20 Mars 2005, Dijon – France. Naturalia Publications, Turriers, pp. 161–172.

Crisci, J.V., O.E. Sala, L. Katinas & P. Posadas. 2006. Bridging historical and ecological approaches in biogeography. – Aust. Syst. Bot. 19: 1–10.

Csuti, B., S. Polasky, P.H. Williams, R.L. Pressey, J.D. Camm, M. Kershaw, A.R. Kiester, B. Downs, R. Hamilton, M. Huso, M. & K. Sahr. 1997. A comparison of reserve selection algorithms using data on terrestrial vertebrates in Oregon. Biol. Cons. 80: 83–97.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology 26: 297–302.

Dunn, G. & B.S. Everitt. 1982. An introduction to mathematical taxonomy. Cambridge University Press, Cambridge.

Faith, D.P. 1992. Conservation evaluation and phylogenetic diversity. Biol. Cons. 61: 1–10.

Gower, J.C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 325–338.

Gower, J.C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857–871.

Humphries, C.J. 2006. Measuring diversity. *In*: E. Leadley & S. Jury (eds), Taxonomy and plant conservation: the cornerstone of the conservation and the sustainable use of plants. Cambridge University Press, Cambridge, pp. 141–161.

Koopowitz, H. 2001. Orchids and their conservation. B.T. Batsford Ltd, London.

Koopowitz, H., P.S. Lavarack & K.W. Dixon. 2003. The nature of threats to orchid conservation. *In*: K.W. Dixon, S.P. Kell, R.L. Barrett, & P.J. Cribb (eds), Orchid conservation. Natural History Publications (Borneo), Kota Kinabalu, pp. 25–42.

Legendre, L. & P. Legendre. 1983. Numerical ecology. Elsevier Scientific Publishing Company, Amsterdam.

Mace, G.M., J.L. Gittleman & A. Purvis. 2003. Preserving the tree of life. Science 300: 1707–1709.

Myers, N. 1988. Threatened biotas: "hot spots" in tropical forests. Environmentalist 8: 187–208.

Myers, N., R.A. Mittermeier, C.G. Mittermeier, G.A.B. da Fonseca & J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 853–858.

Pedersen, H.Æ. 1997a. The genus *Dendrochilum* (Orchidaceae) in the Philippines – a taxonomic revision. Opera Bot. 131: 1–205.

Pedersen, H.Æ. 1997b. *Dendrochilum cootesii*, a new protandrous species from the Philippines. Lindleyana 12: 205–207.

Pedersen, H.Æ. 2001. One new and one rediscovered species of *Dendrochilum* (Orchidaceae) from the Philippines. Lindleyana 16: 231–234.

Pedersen, H.Æ., B. Gravendeel & D. Mudiana. 2004. Three new species of *Dendrochilum* (Orchidaceae) and their phylogenetic positions according to plastid and nuclear ribosomal ITS sequences. Blumea 49: 351–360.

Pedersen, H.Æ., J.J. Wood & J.B. Comber. 1997. A revised subdivision and bibliographical survey of *Dendrochilum* (Orchidaceae). Opera Bot. 130: 1–85.

Pillon, Y., M.F. Fay, A.B. Shipunov & M.W. Chase. 2006. Species diversity versus phylogenetic diversity: a practical study in the taxonomically difficult genus *Dactylorhiza* (Orchidaceae). Biol. Cons. 129: 4–13.

Prendergast, J.R., R.M. Quinn, J.H. Lawton, B.C. Eversham & D.W. Gibbons. 1993. Rare species, the coincidence of diversity hotspots and conservation strategies. Nature 365: 335–337.

Rohlf, F.J. 1998. NTSYSpc. Numerical Taxonomy and Multivariate Analysis System. Version 2.0. User guide. Exeter Software, New York.

Smith, J.J. 1933. Enumeration of the Orchidaceae of Sumatra and neighbouring islands. Repert. Spec. Nov. Regni Veg. XXXII: 129–386.

Sneath, P.H.A. & R.R. Sokal. 1973. Numerical taxonomy. The principles and practice of numerical classification. W.H. Freeman and Company, San Francisco.

Tilman, D., R.M. May, C.L. Lehman & M.A. Nowak. 1994. Habitat destruction and the extinction debt. Nature 371: 65–66.

Vane-Wright, R.I., C.J. Humphries & P.H. Williams, P.H. 1991. What to protect? – Systematics and the agony of choice. Biol. Cons. 55: 235–254.

Weiner, J. & O.T. Solbrig. 1984. The meaning and measurement of size hierarchies in plant populations. Oecologia 61: 334–336.

Williams, P., D. Gibbons, C. Margules, A. Rebelo, C. Humphries & R. Pressey. 1996. A comparison of richness hotspots, rarity hotspots and complementary areas for conserving diversity of British birds. Cons. Biol. 10: 155–174.

Wood, J.J. 2001. *Dendrochilum* of Borneo. Natural History Publications (Borneo), Kota Kinabalu & Royal Botanic Gardens, Kew.

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ORCHID BIOGEOGRAPHY AND RARITY IN A BIODIVERSITY HOTSPOT: THE SOUTHWEST AUSTRALIAN FLORISTIC REGION

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Introduction

Understanding the drivers of orchid diversification and rarity may prove crucial to their conservation. The Orchidaceae is characterised by the presence of mycorrhizal endophytes and a diversity of pollination syndromes (Rasmussen 1995, Jersakova *et al*. 2006). The prevalence of pollination by deceit and the specialised mycorrhizal relationships in some taxa have been implicated in the diversification of the family (Cozzolino & Widmer 2005, Otero & Flanagan 2006). Furthermore, interactions with habitat specialisation may act in concert with these attributes to play a critical role in orchid diversification (Gravendeel *et al*. 2004). The influence of these factors in determining intrinsic rarity in orchids remains poorly known.

Delineation of biogeographic provinces and centres of species rarity give an indication of the broad-scale features responsible for restricting distributions and speciation events in plants (Stebbins & Major 1965, Kessler 2002, Hopper & Goia 2004). Analysis of the factors associated with rarity such as edaphic environment, pollination syndrome and site of mycorrhizal infection could reveal if any strategy has a predisposition to rarity and is limiting distribution at a more local scale. Coupling these two approaches has the potential to provide initial clues into the features influencing orchid speciation and rarity. The Orchidaceae of the South West Australian Floristic Region (SWAFR) are an ideal flora to adopt this approach because of its diversity of pollination syndromes (Hoffman & Brown 1998), diverse mycorrhizal infection patterns (Ramsay *et al*. 1986), intrinsically rare species (Brown *et al*. 1998) and high levels of endemism (Hopper & Goia 2004).

Working within the SWAFR, we tested the following hypotheses (i) the pattern of orchid species richness and endemism is the same as those of the flora in general (ii) biogeographic provinces correspond to climatic and edaphic variation (iii) the incidence of rarity of species varies with site of fungal infection, pollination syndrome and habitat type. The results of this study may act as a guide to future studies of population genetics, speciation and the factors contributing to rarity in the Orchidaceae of the SWAFR.

Method

The distribution of 407 orchid taxa from southern Western Australia was mapped as presence/absence data on a grid of quarter-degree cells using the 13,267 records from the Western Australian Herbarium (PERTH) as of June 2006. Species richness for all quarter-degree grid squares was plotted on a map of southern Western Australia. A similar map of the flora of the SWAFR is presented in Hopper & Gioa (2004). A map of the number of rare taxa per cell was also produced. UPGMA cluster analysis was used to delineate biogeographic provinces for orchids. Presence/absence of taxa at the degree grid square level was used to establish large-scale patterns, while finer resolution was achieved by repeating the analysis at the half-degree scale. Subsequently, the number of taxa endemic to each province was calculated.

All taxa where classified by site of fungal infection, habitat preference and, where possible, pollination syndrome. Categories of fungal infection sites follow those of Ramsay *et al*. (1986). Species were categorised by pollination syndrome based on the published literature and field observations (A.P. Brown, unpublished data). We recognised three pollination syndromes: food reward, food deception, and sexual deception. Species that self- pollinate but also utilise one of these pollination syndromes were included within these categories. The mechanism of attraction in *Corybas*, *Pterostylis* and *Rhizanthella* remain unresolved so these genera were omitted from the analysis of rarity an pollination syndrome. Taxa were classified as occurring in the following habitat types: near-coastal, granite rocks, salt lake margins, swamps, woodlands and variable. Forest, woodland and heathland species were classified together under the woodland category because these habitats usually represent a continuum caused by rainfall and are generally continuous, relatively unfragmented habitats.

We tested if site of fungal infection, pollination syndrome or habitat are associated with restricted distributions, low abundance or a high incidence of rare taxa. Using genera as replicates, Kruskal-Wallis tests were used to test for differences between pollination syndromes and sites of mycorrhizal infection in (i) the mean number of herbarium records (ii) the mean number of occupied grid squares per genus and (iii) the mean proportion of rare taxa. In *Caladenia*, which contains sexual and food deception, means were calculated separately for each subgenus because of multiple evolution of sexual deception (Kores *et al*. 2001).

Results

Species richness was highest in the High Rainfall Province, followed by the South-east Coastal Province, the Transitional Rainfall Province and the Arid Zone (nomenclature of regions follows Hopper & Goia (2004)). Nodes of exceptionally high species richness were high rainfall coastal areas with a diversity of habitats including forests, swamps and coastal woodlands and granite outcrops.

Using degree blocks, broad scale biogeographic provinces corresponded closely to those presented in Hopper & Goia (2004), with the exception of the Brookton province which is only evident in the Orchidaceae. The high rainfall regions and the semi-arid Kalbarri sandplain had the highest level of endemism. While the high rainfall province generally contained a relatively high proportion of rare taxa, the Leeuwin-Naturaliste Ridge and parts of the south-coast had a particularly high proportion of rare species. The Kalbarri region also had an exceptionally high proportion of rare taxa.

The site of mycorrhizal infection showed no significant relationship with incidence of rarity, abundance or distributional extent. Pollination syndrome showed no significant relationship with abundance or distributional extent. However, there was significant variation in the incidence of rare taxa between pollination syndromes (sex average rank = 20.61, food = 15.27, reward = 10.13, F-stat = 4.20, P = 0.03). Using a Mann-Whitney U-test, the significant variation lied between the sexual deception and food reward pollination syndromes (U = 57.0, d.f. 9,8, p = 0.046).

Species with variable habitat requirements had the lowest incidence of rarity (0.01%), woodland (18%) and coastal (25%) areas were intermediate, granite (46%) and swamp (40%) had high incidence and salt lakes (83%) had extremely high incidence of rarity. Woodland and species of variable habitat requirements were more abundant and widely distributed than species occupying the remaining habitats.

Discussion

The Orchidaceae of the SWAFR shows a markedly different pattern of species richness to the flora in general. While the total flora is most diverse in the transitional rainfall province, the orchids have their highest diversity in coastal and lower south-west areas of the high rainfall province. Despite a different pattern of richness, orchids exhibit similar biogeographic provinces to those of the entire flora (Hopper & Goia 2004) with boundaries delineated by rainfall and soil type. There are also clear differences in the regions of endemism. This demonstrates that while broad scale features effect species turnover of orchids in a similar way to the rest of the flora, different local process have been responsible for the accumulation of orchid species and patterns of endemism.

Geographic region and habitat type both influence rarity in orchids of the SWAFR. Rarity was most prevalent in geographic regions with high species richness but particularly so in regions with unique edaphic environments. The naturally fragmented habitats of salt lakes, granites and swamps were most strongly associated with rare species. The prevalence of rare species from these habitats is from rarity of suitable habitat and low colonisation possibilities rather than radiation of taxa through isolation. These results demonstrate the underlying importance of edaphic environment in determining orchid rarity.

While there was no evidence from this study that site of mycorrhizal infection is linked to rarity, sexually deceptive genera showed a higher incidence of rarity than rewarding genera. This could be driven either through the greater fruit set by the provision of a reward (Neiland & Wilcock, 1998, Jersakova & Johnson 2006), or the specialisation associated with sexual deception leaving the orchid vulnerable to a decline in its specific pollinator. The majority of the sexually deceptive genera in the SWAFR are pollinated by parasitoid thynnine wasps (Ridsdill-Smith 1970, Stoutamire 1983), which leaves them further susceptible to changes in the abundance of the pollinator's host (Tscharntke & Brandl 2004).

In this study we have found that there is poor congruence between areas of high species richness and endemism for orchids and angiosperms in general. Thus, in the design of conservation estate, it cannot be assumed that regions important for the flora in general will satisfy the needs of orchid conservation in terms of preserving high species richness and localised endemics. Naturally fragmented habitats are of particular importance. While granite outcrops are reasonably well protected, the other habitats remain under threat. Salt-lakes are a vulnerable habitat due to the narrow band around the lake in which orchids occur and the rising water tables resulting from the removal of over 90% of the original vegetation in the Western Australian wheatbelt (Anon. 2006). Alternatively, swamplands in the SWAFR are generally well protected in the state forests in southern Western Australia, however, the orchid rich swamps of the Swan Coastal Plain have been mostly cleared for agriculture and housing. The effects of a pronounced reduction in rainfall over recent decades (Li *et al*. 2005) remains to be seen.

In deciding management priorities for orchids, researchers should take into account the propensity towards rarity in sexually deceptive species. Due to the specificity of the plant-pollinator relationship, particular attention should be paid to the biology and requirements of the pollinator. In particular, if there are ample sites for recruitment, a direct increase in the abundance of the pollinator may lead to an increase in orchid recruitment. In the longer term, changes in the abundance of a pollinator may precede those of the orchid.

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LITERATURE CITED

Anonymous. 2006. State of the Environment Western Australia – Land – Theme 3. http://portal.environment.wa.gov.au/pls/portal/docs/PAGE/ADMIN_SOE/ADMIN_CONTENT/THEMES/3_LAND.PDF

Brown, A.P., C. Thomson-Dans & N. Marchant. 1998. Western Australia's Threatened Flora. Department of Conservation and Land Management, Perth.

Cozzolino, S. & A. Widmer. 2005. Orchid diversity: an evolutionary consequence of deception? Trends Ecol. Evol. 20 : 487-494.

Gravendeel, B., A. Smithson, F.J.W. Slik & A. Schuiteman. 2004. Epiphytism and pollinator specialisation: drivers for orchid diversity. Philos. Trans. Royal Soc. London *B* 359 : 1523-1535.

Hopper, S.D. & P. Gioia. 2004. The southwest Australian floristic region: Evolution and conservation of a global diversity hotspot. Annual Rev. Ecol. Evol. Syst. 35 : 623-50.

Jersakova, J. & S.D. Johnson. 2006. Lack of floral nectar reduces self-pollination in a fly pollinated orchid. Oecologia 147 : 60-68.

Jersakova, J., S.D. Johnson & P. Kindlmann. 2006. Mechanisms and evolution of deceptive pollination in orchids. Biol. Rev. 81 : 219-235.

Kessler, M. 2002. The elevational gradient of Andean plant endemism: varying influences of taxon-specific traits and topography at different taxonomic levels. J. Biogeogr 29 : 1159-1165.

Kores, P.J., M. Molvray, P.H. Weston, S.D. Hopper, A.P. Brown, K.M. Cameron & M.W. Chase. 2001. A phylogenetic analysis of Diuridae (Orchidaceae) based on plastid DNA sequence data. Amer. J. Bot. 88 : 1903-1914.

Li, Y., W.J. Cai & E.P. Campbell. 2005. Statistical modelling of extreme rainfall in Southwest Western Australia. J. Clim. 18 : 852-863.

Neiland, M.R.M. & C.C. Wilcock. 1998. Fruit set, nectar reward, and rarity in the Orchidaceae. Amer. J. Bot. 85 : 1657-1671.

Otero, J.T. & N.S. Flanagan. 2006. Orchid diversity – beyond deception. Trends Ecol. Evol. 21 : 64-65.

Ramsey, R.R., K.W. Dixon & K. Sivasithamparam. 1986. Patterns of infection and endophytes associated with Western Australian orchids. Lindleyana 1 : 203-214.

Rasmussen, H.N. 1995. Terrestrial orchids from seed to mycotrophic plant. Cambridge University Press, Melbourne.

Ridsdill Smith, J. 1970. The biology of *Hemithynnus hyalinatus* (Hymenoptera: Tiphiidae), a parasite on scarabaeid larvae. J. Austral. Entomol. Soc. 9 : 183-195.

Stebbins, G.L. & J. Major. 1965. Endemism and speciation in the Californian flora. Ecol. Monongr. 35 : 1-35.

Stoutamire, W.P. 1983. Wasp-pollinated species of *Caladenia* (Orchidaceae) in South-western Australia. Austral. J. Bot. 31 : 383-94.

Tscharntke, T. & R. Brandl. 2004. Plant-insect interactions in fragmented landscapes. Annual Rev. Entomol. 49 : 405-430.

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Stephen Hopper is director of the Royal Botanic Gardens, Kew. He has worked on Australian orchid systematics and conservation since 1973. Current interests include generic classification of Australian orchids, and the evolution of southwest Australian orchids.

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GENETIC AND MORPHOLOGICAL VARIATION IN THE *BULBOPHYLLUM EXALTATUM* (ORCHIDACEAE) COMPLEX OCCURRING IN THE BRAZILIAN "CAMPOS RUPESTRES": IMPLICATIONS FOR TAXONOMY AND BIOGEOGRAPHY

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KEY WORDS: allozymes, *Bulbophyllum exaltatum*, Cadeia do Espinhaço, campo rupestre, genetic variability, geographic barrier

Introduction

Bulbophyllum Thouars is a pantropical genus. It is one of the most species-rich genera of the Orchidaceae, with ca. 1.200 species (Dressler 1993). The genus presents myophily (pollination by Diptera) as pollination syndrome. Because orchid species are mainly self-compatible, we expect that fly-pollinated orchids present low variability within the populations and high genetic differentiation among conspecific populations, due to the reduction of the gene flow (Borba & Semir 1998, Borba *et al*. 2001). This could help to explain the high number of species in genera of fly-pollinated orchids, most of them with restricted distribution.

High montane areas of the Southeastern and Northeastern regions of Brazil, mainly in the "campos rupestres" vegetation, are the habitat for a species complex within *Bulbophyllum* sect. *Didactyle*, in which traditionally ca. 15 rupicolous species were recognized. The species of this group are vegetative uniform, being separated exclusively by the floral morphology, mainly lip differences. The main taxonomic problem in this group is the delimitation of *B. involutum*, *B. ipanemense*, *B. longispicatum*, *B. geraense* and *B. warmingianum*.

In the present study, we carried out population genetic studies using isozyme markers in 601 individuals of 33 natural populations, in order to assess the genetic variation and degree of differentiation in some species belonging to this complex. We also performed a morphometric analysis in some of the individuals of the genetic study, using multivariate methods as an attempt to improve species delimitation. Vouchers for each population were deposited in the herbarium of the Universidade Estadual de Feira de Santana (HUEFS).

Results and discussion

The four species studied, considered *a priori* to be *B. exaltatum, B. involutum*, *B. sanderianum* and *B. weddellii*, displayed high genetic variation (H_e 0.086 - 0.404) and a high degree of genetic structure (F_{ST} 0.145 - 0.269) which indicates restricted gene flow. The latter was detected only among populations of *B. exaltatum*, and is probably due to long-distance seed dispersion by wind. However, habitat fragmentation can be a factor even more important for the differentiation of these populations.

In the results of the isozyme analysis, none of the conspecific populations were formed a distinct cluster. Therefore, based on these data, a clear taxonomic delimitation among the four species considered is not possible within this complex. On the other hand, the populations clustered primarily based on the State of origin, which correspond to the two main disjunt areas of campos rupestres in Minas Gerais and Bahia (Fig. 1). The inversion of the relative frequency of the

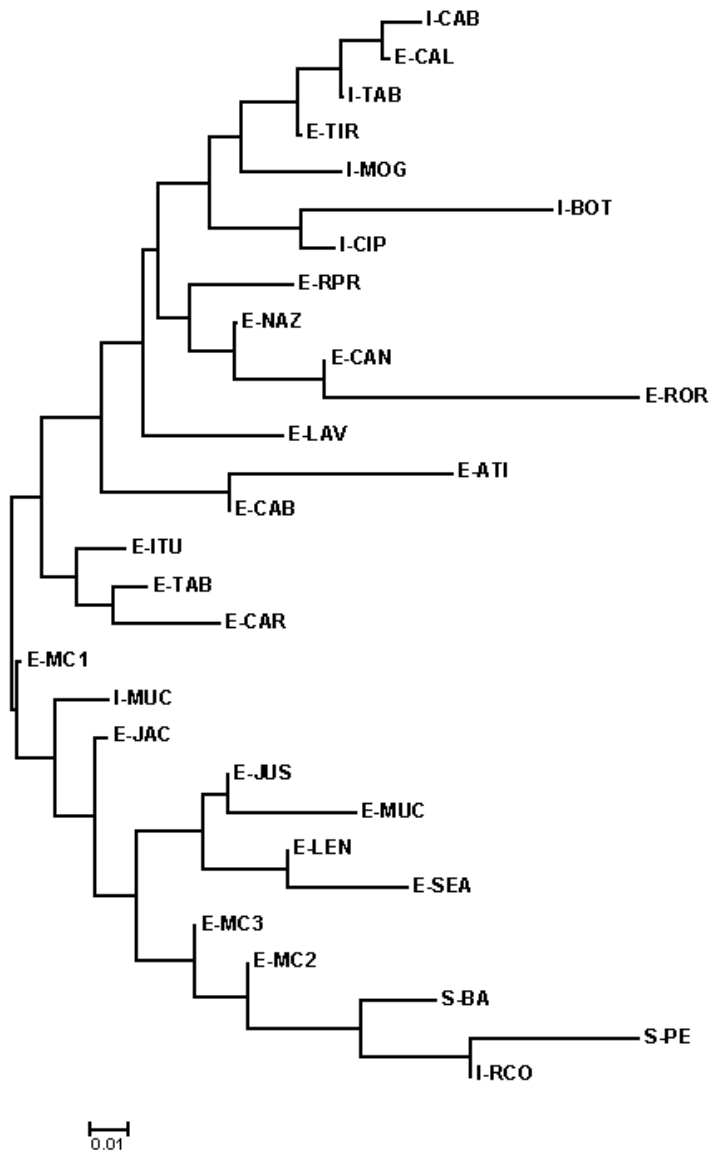

FIGURE 1. Neighbor-joining tree of 29 populations of *B. sanderianum* (S-) (two pops.), *B. involutum* (I-) (seven pops.) and *B. exaltatum* (E-) (20 pops.), based in nine allozymic loci and constructed using the matrix of Nei genetic distances (1978; unbiased genetic distance).

alleles of the locus MDH-1 is probably the main responsible factor for the separation of the two large groups of populations. The first group corresponds to the populations of the states of Bahia and Pernambuco, where the allele MDH-1 100 is most frequent (except for the population E-MC1 of Morro do Chapéu and I-MUC of Mucugê). The second is formed by the populations from São Paulo, Minas Gerais and Roraima States, where the allele 93 is the most frequent (Fig. 2) The genetic differentiation test F_{ST} based on *?* confirms the important participation of this locus in the structuring of the populations for the separation. Perhaps, hybridization events and early differentiation among the taxa have contributed to maintain the high genetic identity among populations, thus generating the observed reticulate pattern of clustering among different species (Fig. 1).

Morphological data suggest that *B. involutum* popula-

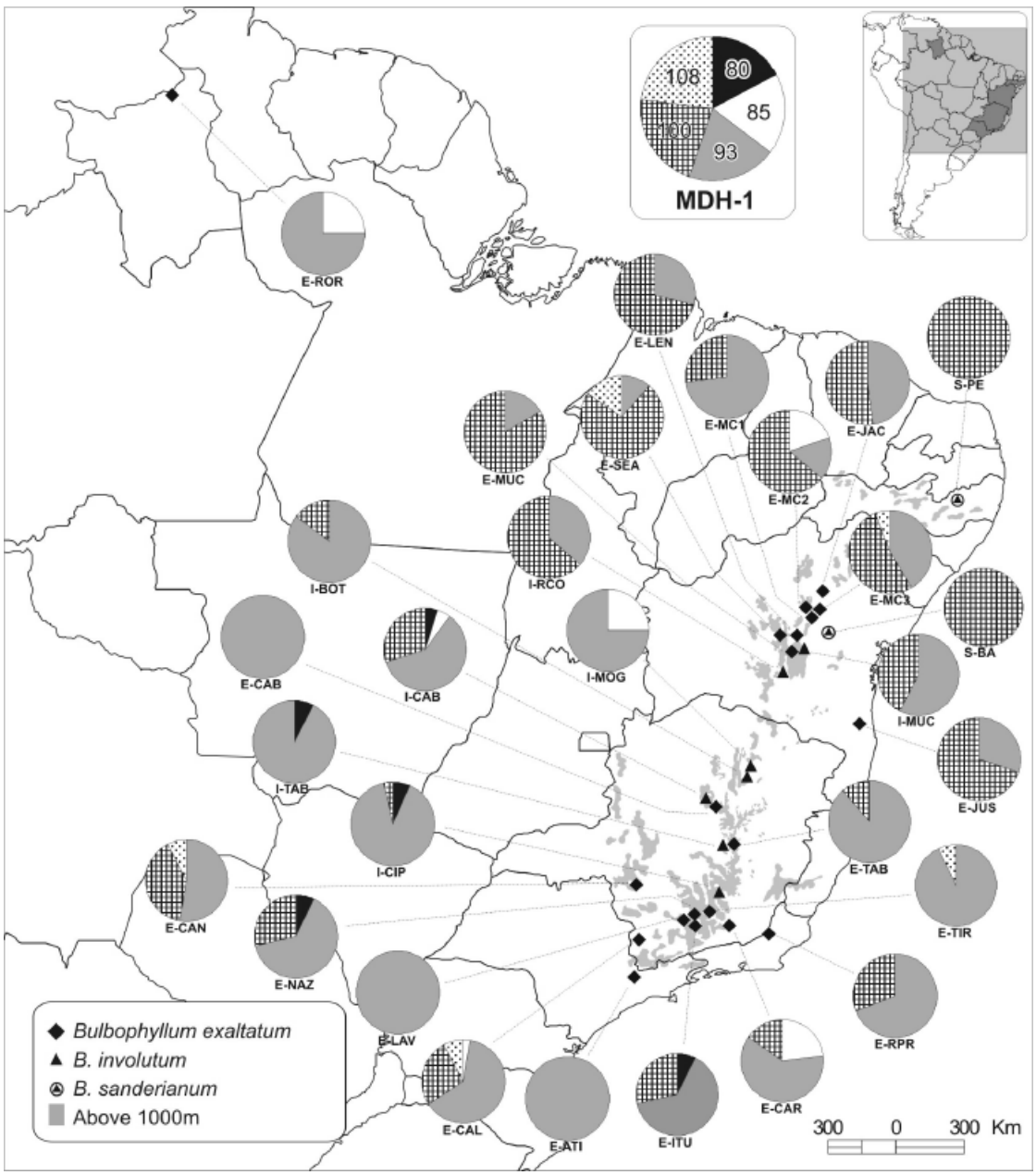

FIGURE 2. Graphic representation of the allele frequencies of the loci MDH-1 in populations of *B. exaltatum* (E-), *B. involutum* (I-) and *B. sanderianum* (S-) studied. Notice the inversion in the relative frequency of the alleles 93 and 100 among the populations from Minas Gerais and Bahia (except I-MUC e E-MC1).

tions in Minas Gerais stand out as a distinct taxon in relation to *B. exaltatum*. However, the populations of both taxa in Bahia State displayed lower differentiation (Fig. 3). In the first axis of the analysis (CVA) we observed more clearly the separation between the populations of *B. exaltatum* from Minas Gerais from Bahia and Roraima plus *B. involutum*, mainly for the smallest size of the lip and larger width of the dorsal sepal in the former. The second axis separates *B. exaltatum* from Bahia plus Roraima from *B. involu-*

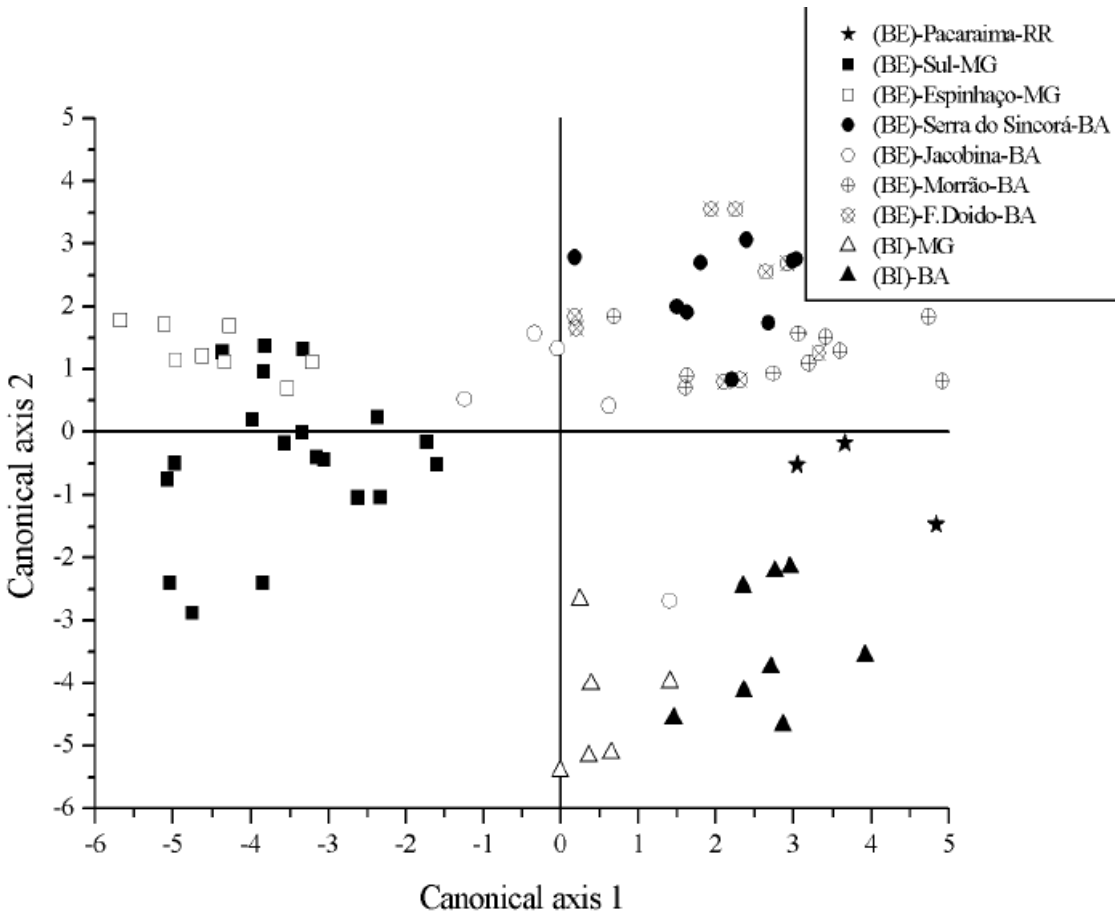

FIGURE 3. Graphic representation of the dispersion on the two first axes of the canonical analysis (CVA), based in 21 morphological characters, from individuals of nine groups of populations of *B. involutum* (BI) and *B. exaltatum* (BE) established *a priori* by geographical areas. Percentual of accumulated variation in the first two axes=71% (axis 1 = 47.3%; axis 2 = 23.7%).

tum. The canonical axes one and two accumulated respectively 47.3 and 23.7% of the variance.

Genetic and morphological data point out that the geographical barriers between Bahia and Minas Gerais, and the larger distance of the Roraima population suggest genetic and morphological differentiation between the populations from these States. The differentiation between the populations from Minas Gerais and the populations from Bahia apparently are related to the main separation of the Cadeia do Espinhaço in two portions. The northern portion is called Chapada Diamantina, and lies entirely in Bahia State, and the south includes the Planalto de Diamantina in Minas Gerais. This geographical separation is a north-south gap of 300 km with lowlands and has been considered as a strong geographical barrier to the migration of campo-rupestre plant species, with apparently great contribution in the differentiation of the plants in the campos rupestres from these areas (Giulietti & Pirani 1988, Harley 1988). Several other disjunctions affecting the geographical distribution in this vegetation has frequently been associated to the genetic differentiation of populations in several groups of plants (Borba *et al.* 2001, Jesus *et al.* 2001, Lambert *et al.* 2006a, 2006b).

Based on genetic, morphological, and reproductive biology of these and other *Bulbophyllum* species studied, we can conclude that all of the populations

considered *B. exaltatum* from Minas Gerais and São Paulo States should be treated taxonomically as a single entity, not divisible even in infraspecific categories. *Bulbophyllum exaltatum* is the oldest name to be applied to the populations from Minas Gerais, generally referred as *B. warminginum*, *B. ipanemense* or *B. geraense* in the literature, thus requiring new synonymies.

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LITERATURE CITED

Borba, E.L. & J. Semir. 1998. Wind-assisted fly pollination on three species of *Bulbophyllum* (Orchidaceae) occurring in the Brazilian campos rupestres. *Lindleyana* 13: 201-218.

Borba, E.L., J.M. Felix, V.N. Solferini & J. Semir. 2001. Fly-pollinated *Pleurothallis* (Orchidaceae) species have high genetic variability: evidence from isozyme markers. Amer. J. Bot. 88: 419-428

Dressler, R.L. 1993. Phylogeny and classification of the orchid family. Portland: Dioscorides Press.

Giulietti, A.M & J.R. Pirani. 1988. Patterns of geographic distribution of some plant species from the Espinhaço Range, Minas Gerais and Bahia, Brazil. Pp. 36-69 *in*: P.E. Vanzolini & W.R. Heyer (eds.). Proceedings of a Workshop on Neotropical Distribution Patterns. Rio de Janeiro: Academia Brasileira de Ciências.

Harley, R.M. 1988. Evolution and distribution of *Eriope* (Labiatae) and its relatives in Brazil. Pp. 71-120 *in*: P.E. Vanzolini & W.R. Heyer (eds.). Proceedings of a Workshop on Neotropical Distribution Patterns. Rio de Janeiro: Academia Brasileira de Ciências.

Jesus, F.F., V.N. Solferini, J. Semir & P.I. Prado. 2001. Local genetic differentiation in *Proteopsis argentea* (Asteraceae), a perennial herb endemic in Brazil. Pl. Syst. Evol. 226: 59-68.

Lambert, S.M., E.L. Borba, M.C. Machado & S.C.S. Andrade. 2006a. Allozyme diversity and morphometrics of *Melocactus paucispinus* (Cactaceae) and evidence for hybridization with *M. concinnus* in the Chapada Diamantina, North-eastern Brazil. Ann. Bot. 97: 389-403.

Lambert SM, Borba EL, Machado MC. 2006b. Allozyme diversity and morphometrics of the endangered *Melocactus glaucescensMelocactus ?albicephalus*.

Patricia Luz Ribeiro first worked with orchids for her undergraduate thesis in Biological Sciences (2001-2003), with the title "The genus *Bulbophyllum* in the Chapada Diamantina, Bahia, Brazil". Since then, she has been working in several projects, such as analysis of intra and inter populational genetic variation of endangered *Cattleya* and *Sophronitis* species from Northeastern of Brazil using allozime markers. During her Master of Science studies (2003-2006), she worked with the "Genetic and morphometric variation on populations of the *Bulbophyllum exaltatum* complex in the Brazilian campos rupestres", supervised by Dr. Eduardo Leite Borba, at the Feira de Santana State University, Bahia, Brazil. She is currently working on the development of DNA barcoding markers in *Cattleya* and *Sophronits* species, supervised by Dr. Cassio van den Berg.

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SPATIAL STRUCTURE OF *PLEUROTHALLIS*, *MASDEVALLIA*, *LEPANTHES* AND *EPIDENDRUM* EPIPHYTIC ORCHIDS IN A FRAGMENT OF MONTANE CLOUD FOREST IN SOUTH ECUADOR

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KEY WORDS: altitudinal range, colonization, phorophyte specificity, phorophyte trunk diameter, seed dispersal, spatial patterns

Orchids are the most diverse family of vascular plants in Ecuador with 228 genera and nearly 4000 species. More than 60% of these species are epiphytes, being *Pleurothallis* R.Br., *Epidendrum* L., *Lepanthes* Sw. and *Masdevallia* Ruiz & Pav., with 472, 358, 314 and 226 species respectively, some of the genera with greater number of epiphytic orchids (Dodson 1994-2003).

Although Ecuador is among those countries with the highest orchid biodiversity in the world, it also has one of the highest rates of deforestation: 1.2% of the country's forests are lost each year (FAO 2005). Extensive deforestation practices currently taking place pose a major threat for the survival of these orchids as they are greatly dependent on the environmental conditions of the forests that sustain them, and the host trees (phorophytes) on which they grow. Thus, understanding of orchid-phorophyte interactions, as well as the patterns of spatial distribution and colonization in secondary succession forests regenerated after deforestation, is essential for the *in situ* conservation. Nevertheless, few studies have been conducted in this field, and scientific basis supporting population reinforcement or reintroduction actions is scarce.

The purpose of this study is to assess the spatial distribution of epiphytic orchids of the above-mentioned genera in an Ecuadorian fragment of secondary montane cloud forest to infer patterns of seed dispersal and colonization. In addition, the effects of phorophyte identity and size on orchid establishment are analyzed. Specifically the questions posed are: Do the distributions of *Pleurothallis*, *Epidendrum*, *Lepanthes*, and *Masdevallia* plants vary in the altitudinal range of the fragment studied? Are there specific patterns in their spatial distribution resulting from seed dispersal characteristics? Do plants of these orchids exhibit any preference over the trees where they grow? Does phorophyte trunk diameter affect the establishment of these orchids? The results presented, although preliminary, provide useful information for orchid management plans.

The study was carried out in a fragment of regenerated forest located on the Loja-Zamora Chinchipe road, on the border of Podocarpus National Park (southern Ecuador). The age of the forest is about 30 years old, and it is characterized by a steep slope (51%), with trees 5-8 m high and lianas that are over 10 m long. Mean annual precipitation is 2700 mm, and annual mean temperature is 15.5 º C (14.4 - 17.5 º C).

A total of nine 10 x 10 m plots were established at 2200, 2230 and 2250 m a.s.l. (three plots in each altitude). All trees (including fern trees), shrubs and lianas of diameter at breast height (DBH) over 1 cm were determined at the genus level, measured and mapped. The census included 1025 vascular plants belonging to more than 70 different genera. *Miconia* Ruiz & Pav. (148 trees), *Nectandra* Rol. ex Rottb. (65 trees), *Clusia* L. (59 trees), *Elaeagia* Wedd. (59 trees) and *Psammisia* Klotzsch (56) were the most frequent genera.

Presence and abundance of all orchids occurring in the first 3 m height were also recorded. In this zone, which corresponds to zone 1 of Johansson's scheme, the microclimatic conditions are relatively constant (Johansson 1974). In total 2798 orchids belonging to 12 genera were identified. Although it is difficult to make comparisons between different researches

TABLE 1. Distribution of *Epidendrum*, *Pleurothallis*, *Lepanthes* or *Masdevallia* orchids on their respectives host trees in a secondary montane cloud forest in South Ecuador. For each orchid genus, frequency of host trees (first column) and frequency of orchid individuals on the different host tree genera (second column) are shown.

Host genus	*Epidendrum*		*Pleurothallis*		*Lepanthes*		*Masdevallia*	
	nº of trees	nº of orchids	nº of trees	nº of orchids	nº of trees	nº of orchids	nº of trees	nº of orchids
Abuta Aubl.	1	3	-	-	-	-	-	-
Alchornea Sw.	-	-	1	18	-	-	-	-
Alzatea Ruiz & Pav.	-	-	1	1	-	-	-	-
Aniba Aubl.	-	-	1	2	-	-	-	-
Anthurium Schott	1	7	-	-	-	-	-	-
Ardisia Gaertn.	2	10	1	5	-	-	-	-
Axinaea Ruiz & Pav.	-	-	2	2	-	-	1	2
Calyptranthes Sw.	1	1	3	16	-	-	-	-
Cinchona L.	1	7	-	-	-	-	-	-
Clethra L.	-	-	1	1	-	-	-	-
Clusia L.	8	28	2	2	3	4	1	2
Cyathea Sm.	2	2	1	21	-	-	-	-
Endlichera C. Presl	1	18	-	-	-	-	-	-
Elaeagia Wedd.	4	12	5	10	5	6	3	8
Eugenia L.	-	-	-	-	3	5	1	1
Faramea Aubl.	1	3	-	-	-	-	-	-
Ficus L.	-	-	-	-	2	2	-	-
Graffenrieda DC.	2	2	2	2	2	8	-	-
Guarea F. Allam. ex L.	-	-	1	1	-	-	-	-
Hedyosmum Sw.	1	36	-	-	-	-	-	-
Helicostylis Trécul	2	14	6	28	3	8	2	3
Hydrangea L.	1	3	-	-	-	-	-	-
Hyeronima Allem.	2	6	1	1	1	1	-	-
Mabea Aubl.	1	5	-	-	-	-	-	-
Markea Rich.	-	-	1	2	-	-	-	-
Maytenus Molina	-	-	1	1	-	-	1	2
Miconia Ruiz & Pav.	3	8	7	7	12	25	3	4
Mikania Willd.	1	2	-	-	1	5	-	-
Myrsine L.	2	16	1	6	3	8	2	3
Nectandra Rol. ex Rottb.	1	5	3	7	3	8	3	4
Ocotea Aubl.	-	-	1	1	-	-	1	1
Palicourea Aubl.	2	19	2	2	-	-	2	8
Persea Mill.	-	-	1	1	-	-	-	-
Piper L.	1	2	-	-	1	3	-	-
Psammisia Klotzsch	2	4	5	22	3	4	6	7
Psychotria L.	2	3	-	-	3	4	-	-
Ruagea H. Karst.	-	-	1	1	-	-	-	-
Turpinia Vent.	-	-	1	1	-	-	-	-
Fallen tree	3	6	3	13	4	8	1	4
Unidentified liana	1	4	2	5	2	5	-	-
Unidentified tree	1	13	-	-	-	-	2	3
Total	**50**	**239**	**57**	**179**	**51**	**104**	**29**	**52**

(mainly because the degree of forest disturbance varies), the high number of orchids that we found on the base of the tree trunks contrasts with other studies, which have reported no orchids or low abundance on this zone (Mehltreter *et al.* 2005). One explanation for our results could be that the lower canopy density of young trees, especially in early succession stages, allows a greater passage of light to the lower areas, providing better conditions for the establishment of orchids. According to this hypothesis, light intensity may affect tree colonization by orchids. In any case, the lower section of the tree trunks seems to have a great relevance for orchids in this regenerating forest.

The most abundant orchid genus was *Stelis* Sw. (73.8%), followed by *Epidendrum* (8.5%), *Pleurothallis* (6.4%), *Lepanthes* (3.7%), *Hexisea* Lindl. (2.5%) and *Masdevallia* (1.9%). Orchids were not uniformly distributed in the altitudinal range studied. *Epidendrum* and *Lepanthes* were more frequent and abundant in lower zone of the fragment. Near 60% of the *Epidendrum* and *Lepanthes* plants were observed at 2200 m. On the other hand, the presence of *Pleurothallis* and *Masdevallia* was similar in all the altitudinal range, although their abundance was greater in the higher zone. Thus, the 66.5% of *Pleurothallis* plants and the 48.1% of *Masdevallia* plants were found at 2250 m. Altitude-related microclimatic factors may be partially responsible for this occurrence pattern, although other environmental factors independent of altitude may also play a role.

Epiphytic orchids were found on 325 of the 1025 recorded trees, shrubs and lianas. The most frequent trees in the fragment were also the ones that had the greatest richness and number of orchids. Of the four genera studied, *Pleurothallis* occupied the greatest number of trees (57), while *Masdevallia* was present in only 29 (see Table 1). The average number of individuals per phorophyte was small in all of them (ranging from 4.8 in *Epidendrum* to 1.8 in *Masdevallia*), but the variance was large especially in *Epidendrum* (±34.8) and *Pleurothallis* (±16.3) (Table 2). In order to know how the individuals are distributed among phorophytes, Morisita's index (I_M) (Hurlbert 1990) was calculated considering the phorophyte as sampling unit. According to this aggregation index (Table 2), two different patterns were

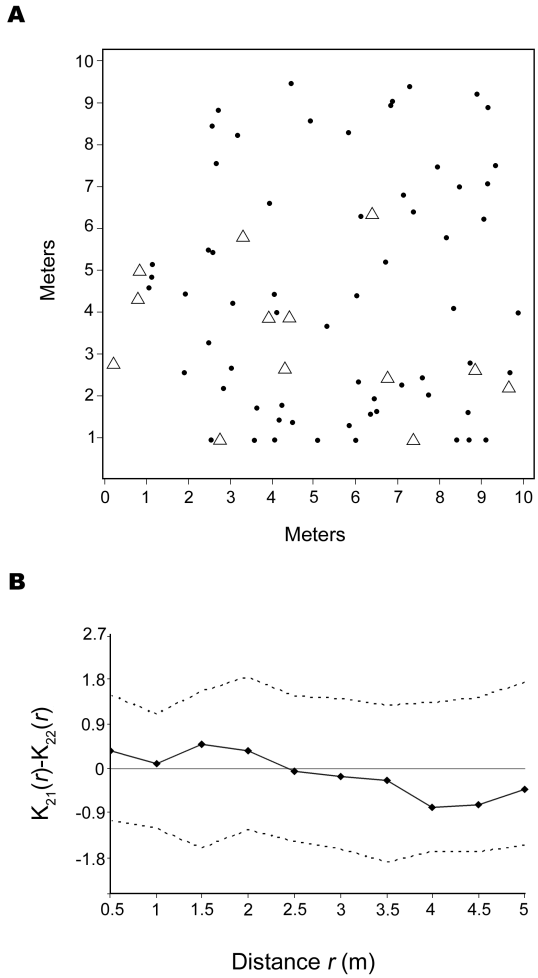

FIGURE 1. **A.** Spatial distribution of trees in plot 1 located at 2250 m. Triangles indicate trees with *Pleurothallis* orchids. **B.** Bivariate point pattern analysis plotting the L_{12} function across distance. Dotted lines represent the confidence interval of random labelling null hypothesis.

detected: *Epidendrum* and *Pleurothallis* plants tended to be clumped (I_M values were significantly different from 1), while *Lepanthes* and *Masdevallia* plants were randomly distributed. Differences in seed dispersal process may explain this result. Thus, the aggregated pattern observed in *Epidendrum* and *Pleurothallis* may be due to limited dispersal ability of their seeds. If this were the case, there would be a higher probability of finding a plant of its same genus in a near-by tree than in a more distant tree. To test this hypothesis, a bivariate point pattern analysis was performed in those plots where the number of phoro-

TABLE 2. Average number of individuals per host tree (mean ± variance) and Morisita´s index (I_M) for *Epidendrum*, *Pleurothallis*, *Lepanthes* or *Masdevallia* orchids in a secondary montane cloud forest in South Ecuador. Values in parentheses are minimum and maximun. ***: P<0.001.

Orchid genus	Individuals per host tree	I_M
Epidendrum	4.8 ± 34.8 (1-36)	2.32***
Pleurothallis	3.1 ± 16.3 (1-21)	2.34***
Lepanthes	2.0 ± 2.4 (1-7)	1.10
Masdevallia	1.8 ± 1.3 (1-5)	1.09

phytes was greater than eight (Diggle 1983, Wiegand & Moloney 2004). For *Pleurothallis*, the values of $K_{21}(r)$-$K_{22}(r)$ were inside the confidence interval of the null hypothesis of random labelling for the range of distances 0-5 m (Figure 1), which means that presence of these orchids is at random. Similar results were obtained for *Epidendrum*. Thus, since there is no contagious distribution between one phorophyte and nearby trees, seed dispersal in *Epidendrum* and *Pleurothallis* is not limited to short distances. This conclusion is also supported by mean distance between phorophytes (4.0 ± 5.6 m for *Epidendrum* and 4.5 ± 6.8 m for *Pleurothallis*), which is not significantly different than the mean distance between all trees in the plots (4.7 ± 2.5 m), and maximum distance to the nearest neighbour (13.2 m for *Epidendrum* and 6.9 m for *Pleurothallis*). Other reasons, such as differences in life cycle or reproductive biology could explain the presence of these two distribution patterns.

No phorophyte specificity was observed for any of the epiphytic orchids included in the study. *Epidendrum* and *Pleurothallis* grew on more than 20 different genera, and *Lepanthes* and *Masdevallia* on more than 10 (see Table 1). Nevertheless, *Epidendrum* was more frequent on *Clusia*, *Pleurothallis* and *Lepanthes* on *Miconia*, and *Masdevallia* on *Psammisia*. Preference patterns in orchids have also been reported by other authors (Migenis & Ackerman 1993, Díaz-Santos 2000, and Trapnell & Hamrick 2006), although the reasons why orchids occur on particular species remain unclear.

The possible effect of phorophyte size on orchid establishment was explored calculating the Spearman's correlation coefficient (r_S) between DBH and orchid abundance for each of these four genera. No relationship was found in any of them (r_S= -0.12 P=0.42 for *Epidendrum*, r_S=0.15, P=0.27 for *Pleurothallis*, r_S=0.09 P=0.52 for *Lepanthes*, and r_S=0.17 P=0.37 for *Masdevallia*), which means phorophyte trunk diameter does not seem to be a crucial factor for orchid colonization. At present, other phorophyte physical characteristics such as bark stability and roughness, and substrate moisture conditions are being investigated.

In conclusion, this study shows the existence of different patterns of presence and abundance depending on each orchid genus. Anyhow, colonization of new trees does not seem to be constrained by limited seed dispersal. Light conditions may be a more important factor for epiphytic orchid establishment than phorophyte identity and size. The abundance of orchids in the lower section of the tree trunks in this regenerating forest is in clear contrast with previous reports made on primary or non-disturbed forests. This outlines the importance of taking into account the different succession states of the forest in which the orchids occur. Finally, although pattern analysis can be helpful in identifying the causes of present spatial structure, additional experimental studies are needed to determine the underlying processes originating these distributions.

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LITERATURE CITED

Díaz-Santos, F. 2000. Orchid preference for host tree genera in a Nicaraguan tropical rain forest. Selbyana 21(1,2): 25-29.

Diggle, P.J. 1983. Statistical analysis of spatial point patterns. Academic Press, London.

Dodson, C.H. 1994-2003. Native Ecuadorian orchids. 4 v. Colina, Quito.

FAO. 2005. State of the world's forests. Food and Agriculture Organization of the United Nations, Roma.

Goreaud, F. & R. Pélissier. 2003. Avoiding misinterpretation of biotic interactions with the intertype K_{12}-func-

tion: population independence vs. random labelling hypotheses. J. Veg. Sci. 14: 681-692.

Hurlbert, S.H. 1990. Spatial distribution of the montane unicorn. Oikos 58: 257-271.

Johansson, D. 1974. Ecology of vascular epiphytes in West African rain forest. Acta Phytogeogr. Suec. 59: 1-136.

Mehltreter, K., A. Flores-Palacios & J.G. García-Franco. 2005. Host preference of low-trunk vascular epiphytes in a cloud forest of Veracruz, Mexico. J. Trop. Ecol. 21: 651-660.

Migenis, L.E. & J.D. Ackerman. 1993. Orchid-phorophyte relationships in a forest watershed in Puerto Rico. J. Trop. Ecol. 9: 231-240.

Trapnell, D.W. & J.L. Hamrick. 2006. Variety of phorophyte species colonized by the neotropical epiphyte, *Laelia rubescens* (Orchidaceae). Selbyana 27(1): 60-64.

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RICHNESS, DISTRIBUTION AND IMPORTANT AREAS TO PRESERVE *BULBOPHYLLUM* IN THE NEOTROPICS

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Introduction

Bulbophyllum is probably one of the largest genera in the orchids with Pantropical occurence, but the distribution is not homogeneous across the world. The Paleotropics is the richest area and there are hundreds of species in Asia (Vermeulen 1991). The genus was described by Thouars in 1822, and the first Neotropical species was described only in 1838 (*B. setigerum* Lindl.) from a plant collected in Guayana by George Loddiges and sent to John Lindley. Until today, one hundred and ten species names were published for the Neotropics, however only *ca.* 70 species could be recognized in five sections supported by phylogenetic studies based on nuclear and chloroplast genome sequence data (Smidt unpubl. data).

Richness is a fundamental measurement of community and regional diversity, and underlays many ecological models and conservation strategies (Magurran 1988). Due to the vast area of the Neotropical region, we know that the sample effort is not consistent throughout the range. Some areas could be richer than others because they are near cities and others could be considered poor in number of species because they are rarely or never sampled. Keeping this in mind, we can use richness estimation to infer the richness from incomplete collections and projecting the probable number of species to be found. The literature about estimation of species richness is extensive (e.g. Colwell & Coddington 1994, Walther & Morand 1998, Hellmann & Fowler 1999, Gotelli & Colwell 2001), and have been used to evaluate global richness of different organisms (e.g. Jarvis *et al.* 2002, Meier & Dikow 2004).

In this study, the richness patterns, relationships of the Neotropical biomes and complementarity analyses of the genus were accomplished by using a GIS framework, considering the proposed phytogeographical areas for the American Continent (Atlantic Rain Forest, Cerrado, Semi-arid, Andean region, Amazon, Highlands Guayana, Mesoamerica, Caribbean and Mexico).

Methodology

SAMPLE DATA - The specimen database was generated during the taxonomic review of Neotropical *Bulbophyllum* species and the information was obtained from ca. 1400 specimens deposited in 65 herbaria in Brazil, Europe and other American countries. All analyses of this study was undertaken using free DIVA-GIS software v. 5.4 (Hijmans *et al.* 2000, 2001) and Arcview GIS 3.3 (ESRI 1999) using the Americas Base Map for Flora Neotropica.

RICHNESS - This study explored the species richness of Neotropical *Bulbophyllum*, by the number of taxa occurring in cells with 1° x 1° size in a grid map. This approach permits us to evaluate where are and the range size of this areas to employ conservation decisions about this taxa. We applied five non-parametric species richness estimators (Chao 1, Chao 2, Jackknife 1, Jackknife 2 and ACE, see Colwell & Coddington (1994) for explanation of these estimators), to know how many species of *Bulbophyllum* are probable to be discovered in the Neotropical region, and which biomes are potentially richer. Each estimator used here presents different assumptions and bias,

but the overall difference between them is how it works with species collected one time (singletons) and two times (doubletons) in the analyses. Many studies with simulation or empiric data have showed that the behavior of these estimators is influenced by the data set, but are of great utility to compare the estimates between different groups in the same environment or compare different environment for the same taxa, e.g. Colwell & Coddington (1994), Meier & Dikow (2004).

COMPLEMENTARITY ANALYSIS - In order to determine optimal locations for *in situ* reserves to conserve maximum species diversity, a study based on species complementarity was carried out using the algorithm described by Rebelo (1994) and Rebelo & Sigfried (1992). The aim was identify grid cells with defined size, which complement each other in terms of species composition. The process is iterative, whereby the first cell is the richest in number of species. The second iteration locates a grid cell that is richest in species not already represented in the first iteration. This iterative process continues until all species have been represented. We computed the minimum number of grid cells needed to capture all 71 Neotropical *Bulbophyllum* species.

PAE ANALYSIS - The parsimony analysis represents a direct tool for searching the most parsimonious arrangement of shared species among areas, aiming to reveal the biogeographical affinities in a hierarchical pattern (Rosen & Smith 1988, Trejjo-Torres & Ackerman 2001, Garcia-Barros *et al.* 2002). This approach was originally called parsimony analysis of endemicity (PAE), developed by Rosen & Smith (1988), and employed in the study of orchids of Caribbean islands (Trejjo-Torres & Ackerman 2001).

The units of comparison that have usually been applied are sites, quadrants or sections of regions, biogeographical areas, or natural geographical areas. In this work, we used well accepted phytogeographical areas, as those discussed by Gentry (1982) for the Neotropics. Among them, nine areas or Biomes (Atlantic Rain Forest, Cerrado, Semi-arid, Andean, Amazon and Guayana Highlands, Mesoamerica, Caribbean Islands and México) were considered.

A presence/absence matrix of the 71 *Bulbophyllum* species (including varieties) was constructed in Nexus Editor Software (Page 2001), where presence was indicated with a `1' and absence with a `0'. Using this program, areas were entered in the place of taxa, while taxa were entered in the place of characters. Once we constructed the matrix, the analyses of parsimony were done using PAUP 4.10 (Swofford 2000). A hypothetical outgroup area with all 0s (no species) was used in the analyses in order to root the trees. Exhaustive search was carried out to look for the most parsimonious trees, which indicate the floristic affinities among studied areas. We obtained a Majority Rule Consensus Trees for equally parsimonious trees founded and assessed the clade robustness using bootstrap proportions with 1000 replicates (Felsenstein 1985).

Results and Discussion

GENERAL DISTRIBUTION - Although there are ca. 1400 specimens available from different herbaria, only ca. 900 could be geographically referenced based on specimen labels, due to the uncertainty or lack of the locality indications. Some species were plotted on map only from type specimens or protologue information because of absence of field sample.

The generic distribution of *Bulbophyllum* in the Neotropics is limited at the South by Rio Grande do Sul (Brazil) and Mexico at the North; at the East it is limited by Pernambuco, Brazil and West by Cordova, Mexico. The historical record of North limit of the genus in Everglades, Florida (Luer 1972) was not confirmed by herbarium specimens.

RICHNESS - The "global" richness through the Neotropics is obviously different from richness of any political boundaries, and the richness of a particular country needs to be evaluated in another scale with smaller grids than those used in this work. According to our results, the highest number of Neotropical *Bulbophyllum* species can be found in Southeast Brazil (22° S - 42° W) more specifically in the contact areas between Cerrado and Atlantic Rainforest (fig. 1). Among the biomes or phytogeographical regions considered, the greatest richness were reported for South American Cerrado biome (35 spp) followed by Atlantic Rainforest (31 spp), and Andean region (17 spp) (tab. 1). Despite the low richness, three species are endemic to Mexico, while the

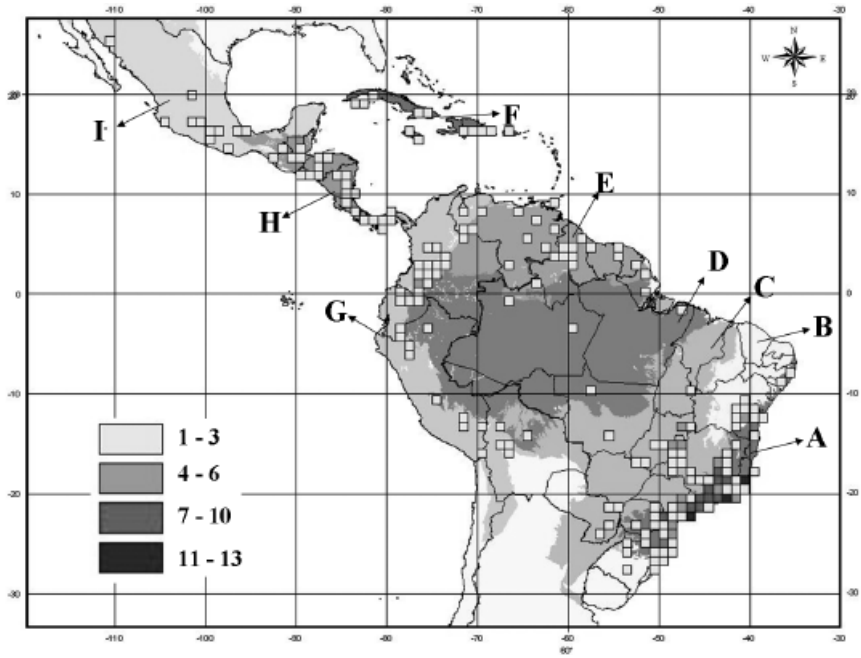

FIGURE 1. Species richness of *Bulbophyllum* data set plotted into grid cells of equal area (ca. 12,000 Km^2, the 1º x 1º Flora Neotropica grid). Values are represented by the number of species present in each cell and the phytogeographical areas by the follow letters: A. Atlantic Rain Forest, B. Semi-arid, C. Cerrado, D. Amazon, E. Guayana Highlands, F. Caribbean Islands, G. Andean, H. Mesoamerica, I. Mexico.

Central America and Caribbean Islands did not present any endemic species.

Considering the estimation of global richness in the Neotropics, the indexes applied indicate that, until today, 50-90% of *Bulbophyllum* species were collected (tab. 1). Although different estimations have been obtained among the biomes, once the richness estimators seem to be sensible to poorly sampled areas and positive biased with highly collected areas, some regional richness patterns for different phytogeographical regions can be explained.

In general, according to the richness estimation analysis, the Cerrado and the Atlantic Forest have the largest number of species and also great amount of collections too, probably being the best sampled areas. In this context, the Andean region is supposed to be one of the richest areas rather than other sites with more samples and species collected. Although only 17 species have been registered, the quantitative estimators Chao1 and Chao2 indicate more than 40 species to be discovered in that region, probably due to the effect of few collections (and consequently more single and doubletons) for the estimations.

A pattern that emerges from the *Bulbophyllum* diversity seems to be the affinities of this genus with outcropping mountain habitats. The Brazilian southeast mountain area presents half of the collected species for the Neotropics, constituting the richest vegetation in species of *Bulbophyllum* for the American continent. The conglomerate of mountains considered in this work extends from Rio de Janeiro to Bahia State and do not present a unique geological origin, being usually divided in three blocks: 1- the adjacent mountains of the National Park of Itatiaia on the south portion, 2- mountains adjacent to Serra do Cipó in the central area, in Minas Gerais State and 3- Chapada Diamantina in Bahia State in the north. Each of these portions of mountains is in contact with different biomes, especially Cerrado and Atlantic Forest.

The low richness found in other ecosystems can be explained by different reasons. In the Amazon few collections have been carried out, except around Manaus - Belém area. In fact, lots of mountain formations in this phytogeographical area have never been explored for plant collection, being this place probably the most suitable to find *Bulbophyllum* species.

TABLE 1. Species richness estimation for *Bulbophyllum* using five estimator indexes for different phytogeographical areas in the Neotropics.

Estimator	Richness (S)	Observation	Chao-1	Chao-2	Jackknife-1	Jackknife-2	ACE
Neotropics	71	888	137	122	93	115	73
Cerrado	34	316	41	39	42	51	35
Atlantic Rain Forest	32	240	47	43	43	53	33
Mountains of Southeastern Brazilian	30	302	40	37	39	47	32
Andes	17	69	64	40	26	35	19
Semi-arid	10	90	14	12	13	16	12
Guayana Highlands	9	35	21	15	13	17	16
Mexico	7	39	8	7	9	10	8
Amazon	4	9	6	5	5	6	7
Caribbean Islands	4	32	4	4	4	5	5
Mesoamerica	2	50	2	2	2	2	-1

Other poor region for richness of this group is the Semi-arid, one of the less adequate biomes for Orchidaceae, due to low air humidity and rainfall. In accordance to the dataset the relative high observed and estimated richness in this biome is due to species sampled in interior mountain formations with humid forest growing around high altitude places.

A low richness was detected in Northern South America to Mesoamerica and in Caribbean islands, where mainly species of section *Bulbophyllaria* Rchb.f. (bifoliated plants with thickened inflorescence, known as "rat-tail orchid") are present. Finally, Mexico presents low richness but a relatively high number of endemic species growing especially in Oak forest and in high altitude places too.

PAE ANALYSIS - The parsimony analysis of endemicity, with the nine phytogeographical regions considered for the Neotropics using shared *Bulbophyllum* species, produced seven equally most parsimonious trees (fig. 2) with: tree length = 82, Consistency index (CI) = 0.8537, Retention index (RI) = 0.7600. A strong floristic relationship was detected between: (1) Cerrado, Atlantic Rain Florest, Semi-arid and Andean biomes and (2) Amazon and Guayana Highlands, Mesoamerica, Caribbean and Mexico. The other Neotropical biomes do not have any *Bulbophyllum* species or are considered within the broader concepts used here to avoid small areas with few collections.

COMPLEMENTARITY ANALYSIS - According to our results thirty five main areas were identified using complementarity analysis and considered as prioritaire for the conservation of total diversity of *Bulbophyllum* in the Neotropics (Fig. 3).

The eight most important areas in the map encompass 54% of the 71 species considered in this study.

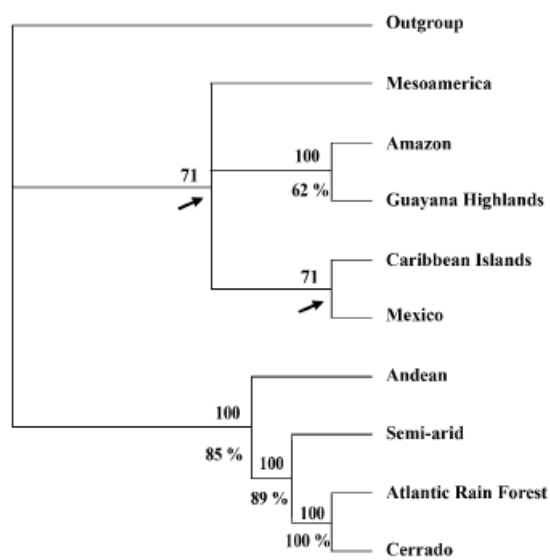

FIGURE 2. Relationship between Neotropical phytogeographical areas using Parsimony Analysis of Endemicity of *Bulbophyllum* species. The value above the branch is the Majority-Rule Consensus index of the seven most parsimonious trees and bootstrap support is below the branches. Nodes with less than 50% of bootstrap support are indicated with arrows.

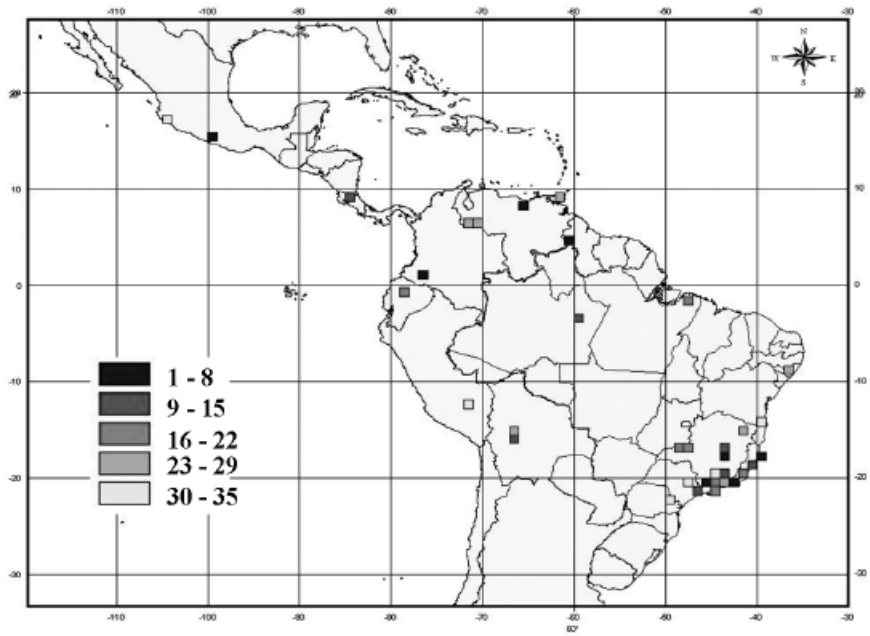

FIGURE 3. The minimum set of areas for the conservation of all Neotropical *Bulbophyllum* species included in the data set according to Complementarity analysis. The values represent an order of importance between the areas.

As expected, four of eight grids coincide with areas of high species richness identified in fig. 1, which indicates that each of the high diversity areas has a distinct species composition. Some of these areas are located in previously proposed Hotspots for other plant and animal groups in the American continent (Meyers *et al.* 2000).

Conclusion

One of the central theoretical tasks of conservation biology is to prioritize places on the basis of their biodiversity value and to devise management strategies to conserve biodiversity in these places (Meyers *et al.* 2000). This study shows how some tools can be useful for the fast identification of priority areas. However, we have to be aware of some problems when we accomplish studies of this nature: 1. underestimation of the richness, once we know there are very few collections in herbaria; 2. use of only herbarium material (data of literature and personal communications are very useful information, but uncertain and non reproductible); 3. there is not a consensus about which estimator should be used and sometimes the results among them are conflicting and; 4 - the most important: we do not know if the collected places are still preserved.

For those reasons, a program for conservation *in situ* based exclusively in this type of data set could be a critical point and need to be observed in future political decisions concerning protected areas. We can exemplify practical situation based on field observation during this investigation:

1. Well collected places but actually strongly disturbed such as inland forests in Jaguariaíva, Paraná State, a historical site for plant collections. This was an important area in the complementarity analysis with several taxa and some endemic species in a grid cell. Nowadays the advance of *Pinus* forests for wood exploration seems to have been causing strong disturbance on original vegetation, bringing a large number of species to become seriously vulnerable.
2. Rare and endemics species. There are lots of species considered rare, but this is due to lack of knowledge. For example, an outcropping rock mountain area in Bahia State, known as Chapada Diamantina was recently investigated about *Bulbophyllum* occurrence (Ribeiro et al. 2005), increasing the number of species from five to twelve in two years of study.

In this sense, another topic that should be pointed is the importance of distinguishing the regional definition (endemism) from areal definition (range restriction), (Peterson & Watson 1998). *Bulbophyllum manarae* Foldats is an example of species described for Venezuela and is recently found in Northeast Brazil. In richness analyses of Brazilian species it might be considered endemic of an area as well as for analyses for Venezuela. Considering the whole of the Neotropical range it will be a widely distributed species showing the relationship among the mountains chain of Southeast Brazil and the Andean mountains.

The occurrence and richness patterns described here for the *Bulbophyllum* records were very similar to those for other organisms (e.g. butterflies, Brown 1987) and was described for many plant groups before (e.g. Prance 1987, Giulietti & Pirani 1988, Knapp 2002), but it is still little explored and discussed in Orchidaceae.

To understand the diversity patterns of the orchid family in the Neotropical region is necessary to carry out investigations with similar approach exploring different taxa. Once the orchids are clearly a monophyletic family, the study of groups with different biological and ecological features, such as preference of habit and habitats, dispersive potential and pollination strategies could bring new insights about the diversification of orchid family in the American Continent.

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LITERATURE CITED

Brown, K.S. 1987. Biogeography and evolution of Neotropical butterflies. In: Withmore, T.C. and Prance, G.T. Biogeography and Quaternary history in tropical America. Clarendon Press. Oxford. p. 66-99.

Colwell, R.K. & J.A. Coddington. 1994. Estimating terrestrial biodiversity through extrapolation. Philos. Trans. R. Soc. Lond. B. 345: 101–118.

ESRI. 1999. ArcView®GIS 3.2a. Environmental Systems Research Institute, Inc. New York.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evol. 39: 783-791.

Garcia-Barros, E., P. Gurrea, M.J. Lucianez, J.M. Cano, M.L. Munguira, J.C. Moreno, H. Sainz, M.J. Sanz & J.C. Simón. 2002. Parsimony analysis of endemicity and its application to animal and plant geographical distributions in the Ibero-Balearic region (western Mediterranean). J. Biogeogr. 29: 109-124.

Gentry, A.H. 1982. Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic flutuations, or an accient of the Andean orogeny? Ann. Missouri Bot. Gard. 69 (3): 557-593.

Giulietti, A.M. & J.R. Pirani. 1988. Patterns of geographic distribution of some plant species from Espinhaço range, Minas Gerais and Bahia, Brazil. *In*: P.E. Vanzolini& W.R. Heyer (eds.). Proceedings of a workshop on Neotropical distribution patterns. Academia Brasileira de Ciências, Rio de Janeiro.

Gotelli, N.J. & R.K. Colwell. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecol. Letters 4: 379-391.

Hellmann, J.J. & G.W. Fowler. 1999. Bias, precision, and accuracy of four measures of species richness. Ecol. Applic. 993: 824-834.

Hijmans, R.J., L. Guarino, M. Cruz & E. Rojas. 2000. Computers tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. Plant Genet. Res. Newsl. 127: 15–19.

Hijmans, R.J., M. Cruz, E. Rojas & L. Guarino. 2001. DIVA-GIS, Version 1.4. A geographic information system for the management and analysis of genetic resources data. Manual. International Potato Center, (CIP), Lima, Peru. http://www.cipotato.org/gis/; verified 5 October 2006;

Jarvis, A., M.E. Ferguson,D.E. Williams, L. Guarino, P.G. Jones, H.T. Stalker, J.F.M. Valls, R.N. Pittman, C.E. Simpson & P. Bramel. 2002. Biogeography of wild *Arachis*: assessing conservation status and setting future priorities. Crop Sc. 43(3): 1100-1108.

Knapp, S. 2002. Assessing patterns of plant endemism in Neotropical uplands. Botan. Rev. 68 (1): 22-37.

Luer, C.A. 1972. The native orchids of Florida. The New York Botanical Garden. W.S. Cowell Ltd, Butter Market, Ipswich, England.

Magurran, A.E., 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, U.S.A.

Meier, R. & T. Dikow. 2004. Significance of specimen dataset from taxonomic revision for estimating and mapping the global species diversity of invertebrates and repatring reliable specimen data. Conserv. Biol. 18 (2): 478-488.

Meyers, N., R.A. Mittermeier, C.G. Mittemeier, G.A.B. Fonseca & J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 863-858.

Page, R.D.M. 2001. NEXUS data editor, v. 0.5.0. (http://taxonomy.zoology.gla.ac.uk/rod/NDE/nde.html - Accessed September 2006)

Peterson, A.T. & D.M. Watson. 1998. Problems with areal definitions of endemism: the effects of spatial scaling. Diversity and Distributions 4: 189-194.

Prance, G.T. 1987. Biogeography of Neotropical plants. In: Withmore, T.C. and Prance, G.T. Biogeography and Quaternary history in tropical America. Clarendon Press. Oxford. p.46-65.

Rebelo, A.G. 1994. Iterative selection procedures: Centers of endemism and optimal placement of reserves. Strelitzia 1: 231–257.

Rebelo, A.G. & W.R. Sigfried. 1992. Where should nature reserves be located in the Cape Floristic Region, South Africa? Models for the spatial configuration of a reserve network aimed at maximizing the protection of diversity. Conserv. Biol. 6: 243–252.

Ribeiro, P.L., E.L. Borba & A.L.V. Toscano-de-Brito, 2005. O gênero *Bulbophyllum* Thouars (Orchidaceae) na Chapada Diamantina, Bahia, Brasil. Rev. Bras. Bot. 28 (3): 423-439.

Rosen, B.R. & A.B. Smith. 1988. Tectonics from fossils? Analysis of reef-coral and sea-urchin distributions from late Cretaceous to Recent, using a new method. *In*: M.G. Audley-Charles & A. Hallman (eds.). Gondwana and Tethys. Special Publication of the Geological Society of London 37: 275–306.

Swofford, D.L. 2000. PAUP: Phylogenetic analysis using parcimony and others methods, version 4.10. Sunderland: Sinquer.

Trejjo-Torres, J.C. & J.D. Ackerman. 2001. Biogeography of the Antilles based on a parsimony analysis of orchid distributions. J.Biogeogr. 28: 775-794.

Walther, B.A., Morand, S. 1998. Compare performance of species richness estimation methods. Parasitilogy 116: 395-405.

Vermeulen, J.J. 1991. Orchids of Borneo vol. 2 *Bulbophyllum*. Toihaan Publishing Company. Sabah. Malaysia. 342p.

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RISK OF EXTINCTION AND PATTERNS OF DIVERSITY LOSS IN MEXICAN ORCHIDS

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RESUMEN. La norma oficial mexicana (NOM-059-ECOL-2001) lista cerca de 200 especies de orquídeas en alguna categoría de riesgo (Extintas, En Peligro de Extinción, Amenazadas y Sujetas a Protección Especial). Construimos una base de datos que incluye la información más relevante para planear las estrategias de conservación de estos taxa (nomenclatura, descripciones e ilustraciones para identificación, datos geográficos de todas las poblaciones conocidas, clima, hábitat, refugios, historia natural y ecología, características poblacionales, factores de riesgo, etc.). La información estará disponible través de la Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México (CONABIO). Con esta base de datos y usando sistemas de información geográfica se detectaron las áreas de concentración de orquídeas en riesgo y como éstas se relacionan con las Áreas Naturales Protegidas y las Áreas Terrestres Prioritarias para la conservación. Se discuten distintos patrones de riesgo, de amenazas y de pérdida de diversidad. Es evidente que los efectos del cambio climático, combinados con el mal manejo de los sistemas en hábitats únicos, constituyen las mayores amenazas. La erradicación de poblaciones de orquídeas en amplias zonas densamente pobladas y afectadas, especialmente en Veracruz y Puebla, pueden representar una pérdida importante de la diversidad genética total de las especies. Se hizo un esfuerzo especial por determinar las tasas de extinción de orquídeas en México. Finalmente, y de manera conjunta con otros biólogos de la conservación se desarrolló un método para evaluar el riesgo de extinción en plantas que está siendo adoptado por las autoridades del país y su uso pretende ser obligatorio en el futuro para la inclusión en las distintas categorías de riesgo.

KEY WORDS: Mexico, extinction rates, species at risk, threats, *in situ* conservation, natural protected areas

The orchid family comprises in Mexico some 1254 species and 21 subspecific taxa (Soto Arenas *et al.* 2007). Notable facts of the Mexican orchid flora are the very uneven distribution of the species in the territory, since about a half of the country is too dry to permit the existence of a single orchid species, and nearly 60% of the species are found in the cloud forests which occupy only about 1-2% of the area of the country (Soto Arenas 1996). A summary of the conservation actions in Mexico can be found in Hágsater and Soto Arenas (1998).

Like in other parts of the world, in Mexico the orchid diversity is being lost. Orchids have intrinsic biological traits that make them vulnerable and the human impact on their populations and habitats is a very important threat that is causing extinctions and significant losses of the species' genetic variation.

The Mexican Official Standard NOM-059-ECOL-2001 lists 183 orchid species in a risk category (none extinct, 16 endangered, 61 threatened, and 106 under special protection). The official list is based on information gathered during the decade of 1980-1990 (Soto Arenas and Hágsater 1990; Soto Arenas 1994), nowadays the conservation status of some taxa has changed, even some taxa have become extinct. Based on the official list, and considering 15 additional taxa that have been reported as extinct or severely in risk in the last years, we constructed a data base that includes the most relevant information in order to plan the conservation strategies of the taxa at risk. The information will be soon available in BIOTICA, the data base of CONABIO, Mexico.

In this work we discuss three different aspects of the information derived from the orchids at risk data base: 1) Evaluation of the importance of current and proposed areas for conservation; 2) determination of

the main risk factors, and 3) estimation of the extinction rates of a rich orchid flora that it is rather well-known compared with many other tropical countries. This will permit us to suggest guidelines and conservation strategies with sound bases.

Materials and methods

DATA BASE. We obtained updated information for the nearly 200 Mexican orchids at risk, including nomenclature, distribution, climate, habitat, natural history, uses, ecology, risk factors, refuges, and possible conservation strategies (e.g. opportunities for *in situ* conservation, presence/absence in natural protected or prioritary regions, the maintenance feasibility outside of the habitat, etc.). This includes a review of literature, herbarium collections, field work, and experience cultivating and propagating the species. The information was captured in a data base using the Biotica 4.0 Information System (CONABIO). For each taxon a bibliographic revision and a list of every known locality, georeferenced, was recorded. This information will be available to the public from CONABIO, except for the precise location of sought-after taxa, subject to intense selective collection. Botanical illustrations, distribution maps, and photos were also included.

POPULATIONS AT RISK AND NATURAL PROTECTED AREAS. The whole of georeferenced populations of all the species at risk was superposed with a digital map of Mexico with its political division in states and showing the official Natural Protected Areas (CONANP, 2006) and the Priority Terrestrial Regions of Mexico (Arriaga *et al.* 2000). This was done using the program ArcView GIS 3.2.

RISK FACTORS. The two most important risk factors for every species of the 200 analyzed were assigned to one of the following categories: habitat conversion/destruction by agriculture, livestock grazing, due to effects of unpredictable climatic events (hurricanes, forest fires, unusual frosts), mining, urbanization, touristic developments, forestry, and charcoal production; habitat degradation by acidic rain, urban warming, by changes in the local hydrology; intrinsic biological factors, selective extraction for the local market, gathering for the international (often past) trade.

A method (MER, SEMARNAT 2002) to determine the risk status was applied to each taxon, since it is now mandatory by the Mexican regulation in order to have an objective assigment of the taxa in the different risk categories. We detected several problems of the method that systematically overestimated the extinction risk. Therefore, with the empirical information in the data base, and together with other conservation biologists, we designed and tested a more objective method to determine more precisely the risk categories. This new method considers a rarity index based in the criteria of Rawinowitz *et al.* (distributional traits, habitat characteristics, intrinsic biological vulnerability; 1986) and an anthropogenic impact index. The method will be available soon from the Mexican Ministry of Environment and Natural Resources (SEMARNAT) and will be mandatory to include any plant species in the Mexican official regulation.

EXTINCTION. It is very difficult to determine if a taxon is really extinct into an area or not. We critically examined every case of Mexican orchid that qualifies as extinct. Two different estimations were done. The first was done visiting all the known previous and verified stations, with searching specifically directed at the particular taxon, and corroborating its later extirpation; this approach gives us a *high confidence* in saying that a species is extinct. The second approach is less exhaustive, since verifying the extirpation of every known population was impossible to do, but there is circumstantial evidence that a particular taxon has disappeared, for example, very little suitable habitat remains, the habitat has been visited and surveyed by our team or other botanists with unsuccessful results, and/or there is an old date of last observation; this approach gives us an indication that the taxon is *probably extinct*. For example, *Plectrophora alata* (Rolfe) Garay was collected in Finca Hamburgo, near Huixtla, Chiapas, in 1935 and it has never been located again in the country. Documented populations exist in similar habitats in Suchitepéquez and Sacatepéquez, Guatemala, some 120 km eastward. Finca Hamburgo has been visited and little suitable habitat remains and *P. alata* has not been located by us or any other botanists that have visited the region. Since the region of the Soconusco, where the plantation is located has suffered extensive clearings and most land has been

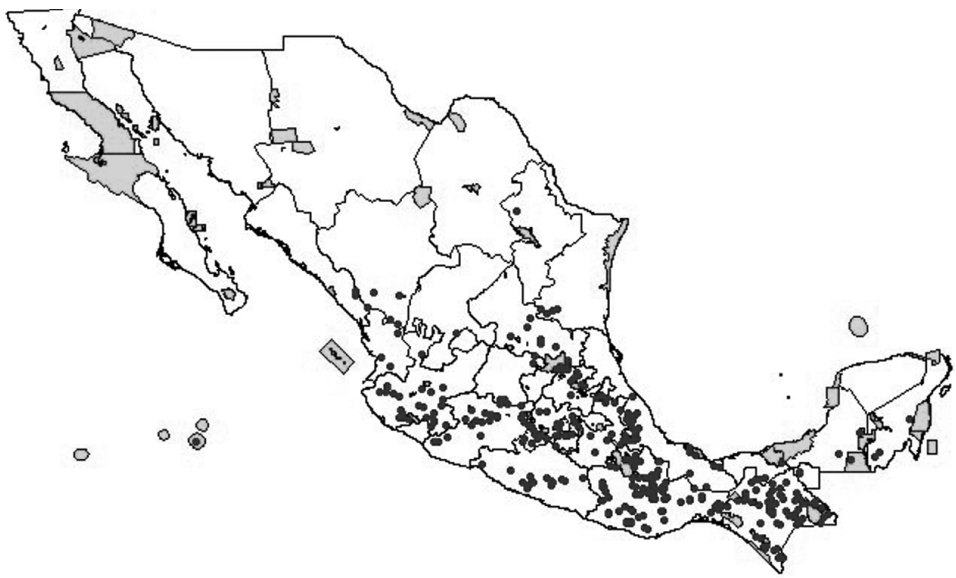

FIGURE 1. Localities of the Mexican orchids at risk (dots) and natural protected areas (in gray). There are 151 areas protected by the Mexican government (CONANP, 2006), but only 43 of them comprise orchid species at risk (15 biosphere reserves, 19 national parks, four natural monuments, and five areas of protection of flora and fauna). Only 120 orchid species at risk (from the 183 included in the NOM-059-ECOL-2001) have populations (at least one) in one of the protected areas. It is evident in the figure that most populations of orchids at risk are found outside of the protected areas, and that regions with a large number of populations of species at risk, as Veracruz, Guerrero, Oaxaca, and Chiapas, are almost devoid of protected areas.

converted into coffee plantations it is *highly probable* that *P. alata* is extinct in Mexico, as suggested by its last record 72 years ago.

We did not consider extinct those species that are very rare, little known, and in which no field work specifically directed to evaluate its populations and habitat has been conducted. For example, *Malaxis lyonnetii* Salazar is known only from one collection near Cuernavaca (Salazar 1997) and another from the nearby locality in Ocuilan (the type of *M. andersoniana* R.González, González Tamayo 2002). It is evidently very scarce, since this area has been well-botanized in the last years, but a rare, inconspicuous plant like this requires a specifically designed search, and this has not been conducted. Additionally, there are yet extensive tracts of suitable habitat that may harbor populations of *M. lyonnetii*. In other probable but excluded cases a single record exists, supposedly collected in Mexico, of a taxon that is very likely not native to the country, and otherwise well-known from other geographic areas (e.g. *Eulophia filicaulis* Lindl. –previously known as *E. ramifera* Summerh.— from Africa, see Salazar and Cribb, in press; or *Maxillaria aurantiaca* A. Rich. & Galeotti, a supposedly Mexican taxon based on a cultivated plant, referable to the Brazilian *Bifrenaria aureofulva* (Hook.) Lindl.).

Results and discussion

Besides the topics discussed in this work, the data base is a source of important information to planning the conservation of the Mexican orchids at risk. For example, it records the regional declining and extirpation of most orchid populations at risk in the densely populated areas of Veracruz and Puebla, and how the same taxa may have healthy populations in Oaxaca. The data base is also an important tool because it gives precise and specific guidelines for *ex situ* conservation programs. It indicates which species are already propagated, which need urgent actions, which present particular difficulties for propagation, which may be traded in the international and national market, among other issues.

POPULATIONS AT RISK AND NATURAL PROTECTED AREAS. Only 120 of the nearly 200 Mexican orchids at risk have populations located inside the System of Natural Protected Areas (SINANP; fig. 1). The sys-

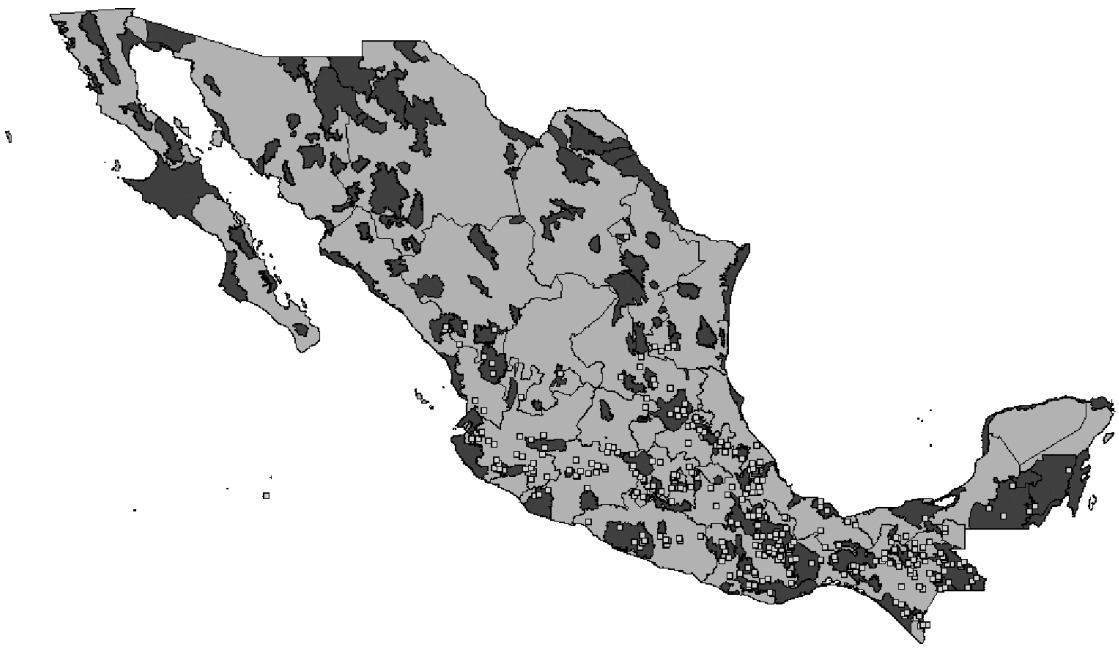

FIGURE 2. Localities of the Mexican orchids at risk (squares) and terrestrial prioritary regions for conservation (dark gray; Arriaga *et al*., 2000). There are 152 prioritary regions but only in 49 have populations of orchids at risk, most of them in the southern part of the country. Of the 183 species at risk included in the NOM-059-ECOL-2001, 171 have populations in the prioritary regions; which indicates that these regions were appropriately selected.

tem is insufficient to fulfill the strategy of conservation *in situ*. Mexican orchid alpha diversity (the diversity into the sites) is rather high in some areas, but at national scale beta diversity (the diversity between sites) is much more important. The biological diversity is widely distributed and it is being maintained by the high environmental heterogeneity, that is, well-defined endemism areas and high beta diversity, as recently stressed in an orchid diversity analysis of the State of Oaxaca (Soto Arenas & Salazar 2004). This means that in order to maintain the biodiversity of the country, the System of Natural Protected Areas must encompass different habitats in different biotic provinces. It is evident that natural protected areas are absent, or almost, in cloud forested regions and in some biotic provinces, like the Sierra Madre del Sur, which has the higher proportion of endemics.

The idea that the present natural protected areas are not sufficient to maintain the biodiversity of the country is not new. In consequence, government agencies and conservationist groups recognized priority terrestrial regions, somewhat equivalent to "hotspots" (regions that harbor biodiversity of global significance, but which are highly threatened). The priority areas attempt to serve the SINANP as a framework when considering the incorporation of new areas, but little effort have been done to date to convert priority regions in protected areas. As 171 orchid species at risk (more than 90% of the whole) have populations inside the priority regions (fig. 2), it is evident that these areas were properly selected and it must be an important task to guarantee that they can maintain their biodiversity. Certainly, the maintenance of orchid species in protected areas is not secured with an official decree, and much work has to be done to provide natural protected areas with management plans and surveillance; however, their official decree is an important first step.

RISK FACTORS. We determined for each one of the 183 orchid species in the NOM-059-ECOL-2001 the two main risk factors (for three species no risk factors are known, for 11 species only a single risk factor is evident). This can be summarized as follow: In 111

species the intrinsic biological factors make then very vulnerable (high habitat specificity, hyperdispersed populations, particular life history, etc.). In 108 species one of their two major risks is the habitat destruction due to agricultural activities, coffee culture being by far the most important. In 49 species the cattle ranching as one of their major risks, with only one case due to goats grazing. In 32 species their habitat have been lost due to forest fires linked to extreme climatic conditions thought to be result of the climatic change. The collection of specimens for the local market is a major risk for 28 species and 21 species have it or had in the past, the collection of specimens for the international horticultural market. Forestry is a threat for 17 species. Four species are at risk due to urban or touristic developments. In three taxa the habitat conversion is the result of the activities to produce charcoal. Two species are at risk due to the industrial, extractive activities and mining. Two species are at risk due to degradation of their habitat because it is located too close to cities with acid rain and urban warming. In two species the plants have suffered severe defoliation and tissue damage by unusual, extremely severe frosts thought to be a result of the climatic change. In one species the habitat has been transformed by the change in the local hydrology of the station.

It is evident that intrinsic factors that make orchid species potentially vulnerable interact with anthropogenic factors that combined put them at risk. The most important is agriculture, cattle raising, and surprisingly the forest fires thought to be result of climatic change, since fires in communities like the mountain rainforest were very unlikely under previous conditions. These threats are followed by the collection to supply the demand of orchids as adornment or for horticulture. Although international trade with wild specimens is probably insignificant nowadays, it was important in the past and was the major threat for taxa like *Laelia anceps* subsp. *dawsonii* (J.Anderson) Rolfe, *Phragmipedium exstaminodium* Castaño, Hágsater & E.Aguirre, or *Rossioglossum grande* (Lindl.) Garay & G.C.Kenn., and the cause of their present endangered status. Collection of wild orchids to be sold in the local Mexican markets has been stressed as a very important threat for species like *L. speciosa* (Kunth) Schltr., *Barkeria scandens* (La Llave & Lex.) Dressler & Halb., *Oncidium tigrinum* La Llave & Lex., *Prosthechea karwinskii* (Mart.) Soto Arenas & Salazar, *P. vitellina* (Lindl.) W.E.Higgins, and many others (Hágsater *et al.* 2005).

TABLE 1. Orchids extinct in Mexico. The following list includes those orchid species whose all known wild populations have been extirpated and that extinction has been verified by field work. Those species marked with an asterisk* were found only in the elfin forest-mountain rainforest of the region of Montebello, Chiapas. The year on the column of the right is the last date in which a wild specimen was seen or alternatively, when the last patch of suitable habitat disappeared.

Anathallis oblanceolata (L.O.Williams) Solano & Soto Arenas	1987
**Cochleanthes flabelliformis* (Sw.) Schultes & Garay	1977
**Dracula pusilla* (Rolfe) Luer	1998
**Dichaea tuerckheimii* Schltr.	1998
**Epidendrum culmiforme* Schltr.	1998
**Epidendrum pansamalae* Schltr.	1981
**Epidendrum tziscaoense* Hágsater	1998
**Eriopsis wercklei* Schltr.	1975
**Jacquiniella gigantea* Dressler, Salazar & García Cruz	1998
Laelia gouldiana Rchb.f.	before 1888
**Lepanthes guatemalensis* Schltr.	1998
**Lepanthes minima* Salazar & Soto Arenas	1998
Lepanthes nigriscapa R.E.Schult. & G.W. Dillon	1936
**Lepanthes stenophylla* Schltr.	1998
**Lepanthes yunckeri* Amex ex Yunck.	1998
**Lycaste dowiana* Endres & Rchb.f.	1998
**Lycaste lassioglossa* Rchb.f.	1985
**Platystele caudatisepala* (C.Schweinf.)	1998
Rossioglossum williamsianum (Rchb.f.) Garay & G.C.Kennedy	1998
**Sigmatostalix guatemalensis* Schltr.	1998
**Specklinia samacensis* (Ames) Pridgeon & M.W.Chase	1998
**Trichosalpinx trachystoma* (Schltr.) Luer	1973

Extinction. Table 1 enlist 22 orchid species (no infraspecific taxa were considered) which we can say with certainty that are extinct in the wild in the country. Three of them, *Anathallis oblanceolata* (L.O.Williams) Solano & Soto Arenas, *Laelia gouldiana* Rchb.f., and *Lepanthes nigriscapa* R.E.Schult. & G.W.Dillon are endemic to Mexico; therefore they

TABLE 2. Orchid species that are probably extinct in Mexico. Those species marked with an asterisk* were found only in the elfin forest-mountain rainforest of the region of Montebello, Chiapas. The year on the column of the right is the last date in which a wild specimen was seen or alternatively, when the last patch of suitable habitat disappeared.

Epidendrum incomptoides Ames	1925
Erycina pumilio (Rchb.f.) N.H.Williams & M.W.Chase	1971
**Habenaria wercklei* Schltr.	1998
Hapalorchis lineatus (Lindl.) Schltr.	1981
Houlletia tigrina Linden ex Lindl. & Paxton	1989
Lyroglossa pubicaulis (L.O.Williams) Garay	1910
Maxillaria oestlundiana L.O.Williams	1984
**Maxillaria praestans* Rchb.f.	2002
Mormodes porphyrophlebia Salazar	1976
Oncidium exauriculatum(Hamer & Garay) R.Jiménez	1989
Oncidium wentworthianum Bateman ex Lindl.	1969
Plectrophora alata (Rolfe) Garay	1935

are extinct on a global scale. Table 2 enlist 12 additional orchid species which are probably extinct in the country, also three of them, *Epidendrum incomptoides* Ames, *Maxillaria oestlundiana* L.O.Williams, and *Mormodes porphyrophlebia* Salazar are endemic. The present extinction rate of Mexican orchids is 1.75% but rises up to 2.71% if the species considered as probably extinct are included. On a global scale Mexico may have contributed with three (or six) already extinct endemic orchid species. From a single species thought to be extinct in 1900, the number increased to about eight species in 1970, and since then it has had an exponential rising, that accelerated in 1998, to reach the present estimate of 34 species.

Greuter (1995) estimated that the extinction rate in Mediterranean higher plants was of 0.15% at the species level, while a rate of 0.14% is derived from the 1997 IUCN Red List of Threatened Plants. While the extinction rates in Mexican orchids are difficult to compare with these global estimates because many species range beyond the Mexican boundaries, 0.24%-0.48% of the endemic Mexican orchids are extinct or probably extinct. Extinction rate in Mexican orchids seems to be comparatively high, especially if we consider that no orchid species are thought to be extinct in areas like the West Indies and Guyanas, and only one is probably extinct in the Paleartic region. The present orchid extinction rate in Mexico is higher that those estimated a decade ago for Southern Africa (0.21%) and Australia (0.625%), although lower than the 3.6% calculated for the Indian subcontinent (data derived from the regional accounts, IUCN/SSC Orchid Specialist Group 1996).

The extinct species share some common traits, especially distributional and habitat preferences. Of the 34 (certainly and probably) extinct Mexican orchids, 28 are species restricted to the mountain rainforest of Chiapas, 22 of them previously found only in the region of Montebello. Four additional species were narrow endemics previously found in the lower mountain rainforest of little extent in the Pacific slope of Oaxaca and Guerrero (the Pluma Hidalgo area and the base of the Teotepec system). Only two taxa have unique distributional traits. *Laelia gouldiana* is a taxon whose specific status is questionable. It has never been found in the wild, its original range is unknown, and recent molecular data (Soto Arenas and Márquez, unpubl. data) suggest that it is a hybrid between *L. autumnalis* (La Llave & Lex.) Lindl. and *L. anceps* Lindl., two taxa which are not sympatric at present. It is probable that *Laelia gouldiana* is the result of an ancient hybridization event. The other particular case is that of *Lyroglossa pubicaulis* (L.O.Williams) Garay, a terrestrial species known from a tropical area of quarzic, acidic sands in southern Veracruz. This habitat is uncommon in the country and it has been subject to severe human impact due to oil extraction, mining, and livestock raising.

The 32 (certainly and probably) extinct Mexican orchids whose habitat was the mountain rainforest share some things in common. They were strict in habitat preferences, for example found only in primary, particularly humid spots of the forest; all were epiphytes that usually had an extralimital distribution in Mexico, most with only one or two populations in the country which may be termed peripherical stations. However, there is a large variation in other traits, some were common plants, others with hyperdispersed populations, some are showy species subject to collection and trade, others are inconspicuous miniatures unknown in cultivation, etc. In most cases the habitat was also severely impacted by agricultural

activities, coffee culture being the very important threat in each and every case. In the case of the 22 species restricted to the mountain rainforest of the Montebello region, their extirpation was due to forest fires that completely destroyed the habitat. Crown forest fires were previously unknown in such a wet habitat and they were conditioned by the vegetation damage during the severe frosts of the winter of 1997-1998, the extreme drought in the spring of 1998, and the use of fire in the management of agricultural and cattle grazing areas. The extreme climatic conditions dried the epiphytic loads to convert them in a suitable fuel for the crown fires.

Extinction was therefore a gregarious event important in marginal (or of little extension), diverse habitats which were sensitive to the effects of the climatic change, and where bad management practices prevailed. It is therefore prioritary to recognize which habitats are the most vulnerable in order to foresee these gregarious losses. For example, the patches of moist coniferous forest in Coahuila and Nuevo León, surrounded by arid regions could be very sensitive to extreme climatic conditions (wildfires already destroyed large tracts in 1975, near San Antonio de las Alazanas, Coahuila). The relictual lower mountain rainforests of southern Oaxaca and Guerrero have been already listed as very threatened habitats (Soto Arenas 1996). The ravines with evergreen tropical forest near El Tuito, Jalisco, which harbor the only populations of rainforest plants in western Mexico, could be also very vulnerable.

Habitat condition is intuitively less adequate in peripherical populations than in the middle of the species' distribution (Olson *et al.* 2005). Climatic change should cause dramatic reduction in the ranges of many species by eliminating the peripherical, outlying populations. Extinction of the orchids of the mountain rainforest of Chiapas means clearly a contraction of the species' ranges. Although researches have stressed that animal species' responses to climate change are individualistic (Team species 2003), plant communities are assemblages in which some components share a high fidelity to the habitat, and therefore plant species' responses may be similar.

In this work we show data, if circumstantial, that climate change is an orchid threat, perhaps much more dangerous and imminent that previously realized. The social and economic problems that provoke a bad management of the environment combine in a bad way with climatic change to be together, by far, the most important extinction threats in Mexico. Collecting evidence on this combined threats and quantifying its effects are a prioritary task for orchid conservation.

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LITERATURE CITED

Arriaga, L., J. M. Espinoza, C. Aguilar, E. Martínez, L. Gómez & E. Loa (coordinators). 2000. *Regiones terrestres prioritarias de México.* Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México.

CONANP. 2006. Areas naturales protegidas federales de México. Comisión Nacional de Areas Naturales Protegidas. Morelia, México.

González Tamayo, R. 2002. *Malaxis* (Orchidaceae), breve discusion de los rasgos específicos y dos taxones nuevos mexicanos. Ibugana 10(1-2): 67-75.

Greuter, W. 1995. Extinctions in Mediterranean areas. *In*: J. Lawton & R. May (eds.). Extinction rate. Oxford University Press.

Hágsater, E. & M.A. Soto Arenas. 1998. Orchid conservation in Mexico. Selbyana 19(1): 15-19.

Hágsater, E., M.A. Soto Arenas, G.A. Salazar Chávez, R. Jiménez Machorro, M.A. López Rosas & R.L. Dressler. 2005. Las orquídeas de México. Instituto Chinoín, México, 304 pp.

IUCN/SSC Orchid Specialist Group, 1996. Orchids - Status survey and conservation action plan. IUCN, Gland, Switzerland and Cambridge, UK.

Olson, M.E., J.A. Lomelí & N.I. Cacho. 2005. Extinction threat in the *Pedilanthus* clade (*Euphorbia*, Euphorbiaceae), with special reference to the recently rediscovered *E. conzattii* (*P. pulchellus*). Am. J. Bot. 92(4): 634-641.

Rawinowitz, D., S. Cairns & T. Dillon. 1986. Seven forms of rarity and their frequency in the flora of the British Isles. Pp. 182-204 *in*: M.E. Soulé (ed.) Conservation biology, the science of scarcity and diversity. Sinauer, Sunderland, Mass.

Salazar Chávez, G.A. 1997. A new species of *Malaxis*

(Orchidaceae) from Morelos, Mexico. Brittonia 49: 449-451.

Salazar Chávez, G.A. & P.J. Cribb (in press). On the identity of *Eulophia filicaulis* Lindl. (Orchidaceae). Kew Bull.

Soto Arenas, M.A. 1994. Population studies in Mexican orchids. Pp. 153-160 *in*: A. Pridgeon (ed.). Proceedings of the 14th World orchid conference. HMSO, Edimburgh.

Soto Arenas, M. 1996. Regional Accounts: Mexico. Pp. 53-58 in IUCN/SSC Orchid Specialist Group. Orchids - Status survey and conservation action plan. IUCN, Gland Switzerland and Cambridge, UK.

Soto Arenas, M.A. & E. Hágsater. 1990. Algunas ideas acerca de la conservación de las orquídeas mexicanas y un listado preliminar de los taxa amenazados. Pp. 155-172 *in*: J.L. Camarillo & F. Rivera (eds.). Áreas naturales protegidas y especies en extinción. Universidad Nacional Autónoma de México, Mexico City.

Soto Arenas, M.A., E. Hágsater, R. Jiménez Machorro, G. Salazar Chávez, R. Solano Gómez & R. Flores. 2007. Las orquídeas de México. catálogo digital. Instituto Chinoín, A.C., Mexico City. DVD.

Soto Arenas, M.A & G.A. Salazar Chávez. 2004. Orquídeas. Pp. 271-295 *in*: A.J. García-Mendoza, M.J. Ordoñez & M. Briones-Salas (eds.). Biodiversidad de Oaxaca. Instituto de Biología, UNAM-Fondo Oaxaqueño para la Conservación de la Naturaleza-World Wildlife Fund, México City.

SEMARNAT. 2002. Norma Oficial Mexicana NOM-059-ECOL-2001, Protección ambiental-Especies de flora y fauna silvestres de México-Categorías de riesgo y especificaciones para su inclusión, exclusión o exclusión-Lista de especies en riesgo. Diario Oficial de la Federación, 6 de marzo de 2002. México.

Team species. 2003. Climate changes species survival. Species 39: 8.

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INVENTORY OF THE ORCHIDS IN THE HUMID TROPICAL PREMONTANE FOREST ON UCHUMACHI MOUNTAIN, NOR YUNGAS REGION OF LA PAZ, BOLIVIA

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RESUMEN. Para conocer la diversidad e importancia de las orquídeas en el bosque húmedo tropical premontano del Cerro Uchumachi (Nor Yungas, La Paz, Bolivia), se realizó un inventario de orquídeas durante 8 meses (febrero a septiembre de 2006) en un área aproximada a 80 ha, utilizando transectas lineales que ubican la principal en el camino carretero comprendido entre dos comunidades. Se establecieron 95 parcelas de 20 m x 20 m, en las que se efectuaron la recolección, herborización, descripción botánica y taxonomía de las mismas. La búsqueda y recolección de orquídeas en las transectas dieron como resultado valores de: densidad absoluta, densidad relativa, y frecuencia. Se encontraron un total de 2159 orquídeas de 16 géneros y 31 especies, pero se crea que existen más que no han sido identificados por la falta de floración durante el tiempo del estudio. La especie de mayor densidad fue *Epidendrum funckii* con una densidad relativa de 33.72%. Asimismo la especie que se presentó con más frecuencia en las parcelas fue *Pleurothallis xantochlora* con 22.11%.

KEY WORDS: inventory, humid tropical premontane forest, cloud forest, Bolivia

Introduction

A greater of understanding of orchids in unstudied areas contributes to conservation, tourism and ecology in general. To understand the diversity of orchids in the premontane humid tropical forest area of Bolivia, an orchid inventory was conducted over 8 months (February to September 2006) in a cloud forest in northwestern Bolivia.

Description of Study Area

The geography of the region is formed from the eastern slopes of the Andes. The eco-region, called yungas, is characterized by mountain chains with wide slopes and long valleys formed from sedimentary and metamorphic rock. Altitudes range from 400 to 2800 meters above sea level (Morales, 2004).

The study area was comprised of primary and secondary forest. A small house was included in the secondary forest area, with a small plot of maize (*Zea mays*). Secondary forest had a great diversity of species, including tree ferns (*Cyathea amazónica*), "sikilis" (*Inga* sp), walnut (*Juglans boliviana*), "ambaibos" (C*ecropia angustifolia*), and diversity of ferns, mosses and palms. Secondary forest was characterized by a dense understory; there was also evidence of selective logging.

The transition between secondary and primary forest is evidenced by the presence of taller, higher-diameter trees, and a reduced understory due to a reduction of light to the forest floor. Evidence of human activity is also much reduced. This forest is dominated by tree ferns (*Cyathea amazónica*), individuals from the *Lauraceae* family, "espeke" (*Clusia haughtii*), "leche leche" (*Sapium aereum*), and "mata palo" (*Ficus obtusifolia*), that can reach diameters of over 100 cm and account for a large part of the basal area. Other species such as "jaluti" (*Macrounea guianensis*), "gironda" (*Siparuna gesneroiodes*), wild papaya (*Oreopanax* sp) and "suti suti" (*Miconia minutiflora*) are found at densities of one or less per hectare, indicators that they may be under the threat of extinction (Endara, 2001).

The forest soils are variable, with a 0 to 20 cm layer of organic matter. The soil closest to the surface is generally loamy with a predominance of silt (39%), followed by sand (37%) and clay (21%). Soil structure is subangular blocky, friable when moist, with a relatively high organic matter content (6.9%).

Permeability is medium to high. Soil pH is very low (3.84 in $CaCl_2$), and low cation exchange capacity (7.4 cmol/kg) (Villca, 2001).

Ten year average data from an on-site weather station show high average temperatures in January (above 19ºC) and low averages in June and July (15ºC), with registered maximum highs around 25ºC and lows of 15ºC. Total annual precipitation is registered as 2390 mm, with maximum precipitation in the months of December through April (200 to 300 mm per month) and no months with less than 80 mm per month. Relative humidity is 100% at night, and falls as low as 50% during the day.

Methodology

The study covered approximately 80 hectares (ha) of Uchumachi Mountain using linear transects extending out from the principal road between the communities of Carmen Pampa and Chovacollo in the municipality of Coroico, province of Nor Yungas, department of La Paz. Carmen Pampa is located at 16º20'30" South and 67º50'00" West; the study plots ranged in altitude from 1880 to 2975 meters above sea level.

Orchids in 95 evenly spaced plots measuring 20 m by 20 m were collected, preserved and described. The transect plots, representing 4.75% of the 80 ha, yielded values for absolute densities, relative densities and frequencies for this site. Species identification was verified at the Herbario Nacional de Bolivia.

Orchid Density

A total of 2159 individual identifiable orchids from 16 genera and 31 species were found, and the presence of more species is suspected but not identified due to lack of flowers over the collection time (Table 1). This count yields an absolute density of approximately 568 orchids/ha.

The species *Epidendrum funckii* was the most abundant with 728 individuals and a density of 192 individuals/ha, and a relative density of 33.72%. The next group, those with densities between 30 and 100 individuals/ha (and relative densities between 5 and 15%), are *Sobralia yauaperyensis, Sobralia fimbriata, Pleurothallis xanthochlora,* and *Restrepia antenifera.* The remaining 26 species *(Bletia catenulata, Elleanthus hookerianus, Epidendrum carpophorum, Epidendrum incisum, Epidendrum incisum, Epidendrum jajense, Epidendrum Secundum, Estelis* sp1*, Estelis* sp2*, Habenaria sartor, Koellenstenia boliviensis, Maxilaria aggregata, Maxilaria longicaulis, Maxillaria aurea, Notylia boliviensis, Oncidium tigratum, Oncidium mentigerum, Pleurothallis cordata, Pleurothallis heliconioides, Pleurothallis linguifera, Polystachia boliviensis, Scelochilus larae, Sobralia dichotoma, Sobralia dorbigniana, Sobralia* sp*, Sobralia suavolens* and *Zygopetalum intermedium)* had densities of less than 20 individuals/ha, representing 630 of the 2159 individuals identified.

The presence of *Epidendrum funckii* in such great numbers implies that the environmental conditions are greatly favorable for its propagation, especially on road borders where it is adapted to the soil. In addition, the brush along roadsides is cleared twice per year, leaving the ground open to expansion, reducing competition from other plant species, and favoring access to sunlight. The species' sympodial growth character also favors its dispersion.

Orchid Frequency

The most frequent species in the plots was *Pleurothallis xantochlora* with 22.11%, found in 21 of the 117 plots (Table 2). This species has a preference for moist forest areas, a characteristic of the primary and old-growth secondary forest in this study. It also has many flowers per plant which increases the chance of fecundation. It is also found in a variety of habitats, both high in the canopy and on fallen and rotting trunks.

Eight species fall into the intermediate category of frequencies of 5 - 16%: *Epidendrum funkii, Estelis* sp1*, Epidendrum secundum, Sobralia fimbriata, Sobralia yauaperyensis, Maxillaria aurea, Sobralia dichotoma* and *Estelis* sp2. Two species, *E. funckii* and *E. secundum,* are always found in the same places, possibly due to their soil preferences; the other six species were all found along the roadside with the characteristics described above.

The remaining 22 species exhibited frequencies below 5%. The least frequent were *Elleanthus hookerianus, Epidendrum carpophorum, Maxilaria aggregata, Maxilaria longicaulis, Notylia boliviensis, Oncidium mentigerum, Pleurothallis heliconioides* and *Scelochilus larae* with a frequency of 1.05% each.

TABLE 1. Densities of orchids on Uchumachi Mountain, Nor Yungas, Bolivia.

Species	Total plants encountered	Density (plants/ha)	Relative Density (%)
Epidendrum funckii	728	191.58	33.72
Sobralia yauaperyensis	284	74.74	13.15
Sobralia fimbriata	221	58.16	10.24
Pleurothallis xanthochlora	182	47.89	8.43
Restrepia antenifera	114	30.00	5.28
Stelis sp. 1	73	19.21	3.38
Zygopetalum intermedium	61	16.05	2.83
Sobralia sp	54	14.21	2.50
Maxillaria aurea	52	13.68	2.41
Stelis sp. 2	49	12.89	2.27
Sobralia dichotoma	46	12.11	2.13
Bletia catenulata	38	10.00	1.76
Polystachia boliviensis	28	7.37	1.30
Epidendrum secundum	27	7.11	1.25
Habenaria sartor	26	6.84	1.20
Scelochilus larae	24	6.32	1.11
Sobralia dorbigniana	24	6.32	1.11
Pleurothallis linguifera	21	5.53	0.97
Sobralia suavolens	19	5.00	0.88
Oncidium tigratum	17	4.47	0.79
Epidendrum jajense	13	3.42	0.60
Epidendrum incisum	9	2.37	0.42
Koellenstenia boliviensis	9	2.37	0.42
Notylia boliviensis	9	2.37	0.42
Oncidium mentigerum	8	2.11	0.37
Maxilaria aggregata	6	1.58	0.28
Pleurothallis cordata	5	1.32	0.23
Pleurothallis heliconioides	4	1.05	0.19
Elleanthus hookerianus	3	0.79	0.14
Maxilaria longicaulis	3	0.79	0.14
Epidendrum carpophorum	2	0.53	0.09
TOTAL	**2159**	**568.16**	**100.00**

Conclusion

Information about density, frequency, habitat and flowering times is useful for planning for tourism activities, which are growing in importance in this area of Bolivia. This information can be used to create eco-tourist paths through the cloud forest for observation and education. Inventory data is also useful to justify conservation activities as slash and burn agriculture encroaches more and more into these environments. Inventories also increase the potential for preservation of orchid germplasm, and tissue culture can be considered to raise and sell the more marketable species found without creating an imbalance in the ecosystem from which the species originate.

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TABLE 2. Frequencies of orchids on Uchumachi Mountain, Nor Yungas, Bolivia.

Species	No. plots where sp was found	Frequency (plots with sp/total plots) (%)
Pleurothallis xanthochlora	21	22.11
Epidendrum funckii	15	15.79
Estelis sp1	15	15.79
Epidendrum secundum	14	14.74
Sobralia fimbriata	12	12.63
Sobralia yauaperyensis	9	9.47
Maxillaria aurea	8	8.42
Sobralia dichotoma	6	6.32
Estellis sp2	5	5.26
Sobralia suavolens	4	4.21
Epidendrum jajense	3	3.16
Pleurothallis cordata	3	3.16
Pleurothallis linguifera	3	3.16
Bletia catenulata	2	2.11
Epidendrum incisum	2	2.11
Habenaria sartor	2	2.11
Koellenstenia boliviensis	2	2.11
Oncidium mentigerum	2	2.11
Polystachia boliviensis	2	2.11
Restrepia antenifera	2	2.11
Sobralia dorbigniana	2	2.11
Sobralia sp	2	2.11
Zygopetalum intermedium	2	2.11
Elleanthus hookerianus	1	1.05
Epidendrum carpophorum	1	1.05
Maxilaria aggregata	1	1.05
Maxilaria longicaulis	1	1.05
Notylia boliviensis	1	1.05
Oncidium tigratum	1	1.05
Pleurothallis heliconioides	1	1.05
Scelochilus larae	1	1.05
TOTAL	**146**	**100.00**

LITERATURE CITED

Endara, A.R. 2001. Inventario de las especies forestales del bosque húmedo tropical premontano del Cerro Uchumachi sector Carmen Pampa. Tesis De Grado. Universidad Católica Boliviana - Unidad Académica Campesina de Carmen Pampa. La Paz, Bolivia.

Morales, C. B. 2004. Manual de ecología. 2ª Edición. Editorial Instituto de Ecología, Universidad Mayor de San Andrés. La Paz, Bolivia.

Villca H., R. 2001. Evaluación de la erosión hídrica en un sistema agroforestal café (*Coffea arabica*) con sikili (*Inga adenophylla*) bajo dos métodos de control de maleza con chonta y machete en Carmen Pampa. Tesis de grado. Universidad Católica Boliviana - Unidad Académica Campesina de Carmen Pampa. La Paz, Bolivia.

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CONSERVATION POLICIES AND BOTANICAL GARDENS

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EX SITU CONSERVATION OF TROPICAL ORCHIDS IN UKRAINE

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KEY WORDS: Orchidaceae, *ex situ* conservation, living collection, *in vitro* propagation, orchid exhibition greenhouse

The problem of biodiversity conservation of tropical floras having the global ecological, economical and social importance, has already exceeded the boundaries of individual countries. Successful conservation actions require joint efforts of scientists on the local, national, regional, and international levels (Wyse Jackson & Sutherland, 2000, Grodzynsky *et al.*, 2001).

The National Botanical Garden (NBG) of the National Academy of Sciences of Ukraine plays the leading role in *ex situ* conservation and propagation of tropical plants in Ukraine. Diverse studies aimed at protection of rare species of various families of angiosperms, particularly tropical orchids, were carried out at this institution for more than 30 years.

Since many representatives of the orchid family are under threat of extinction in their native habitats in the tropics, these investigations are viewed among the highest priorities of our research agenda. The main factors threatening the natural populations of tropical orchids include (1) large-scale collecting of these plants for commercial purposes as ornamental or medicinal plants, and (2) changes of ecological conditions in natural habitats. These changes are caused by human activities, such as agricultural development of territories for crop cultures, industrial development, road construction, mining, recreation, timber harvesting (especially clear-cutting of forests). These activities result in to deforestation, habitat fragmentation, forest fires, invasions of alien species, and other adverse processes (Averyanov *et al.*, 2000, Cribb *et al.*, 2005, Dunkan *et al.*, 2005).

Taking into account the disastrous rate of degradation of virgin or even semi-natural tropical forests (which are natural habitats of many species) as well as biological peculiarities of orchids (mycosymbiotrophic interactions, prolonged and complicated life cycles, pollination limitation), it often happens that the measures taken to protect orchids in their natural habitats, such as monitoring of population, establishment and management of protected areas, are not sufficient for ensuring their survival in nature. Because of that, *ex situ* conservation of tropical plants shall be viewed, along with *in situ* protection, as an efficient alternative way of biodiversity conservation. It is especially true for tropical orchids, especially those species which are threatened with extinction within their native ranges.

The main activities of NBG in the field of conservation of tropical orchid species *ex situ* include maintenance of living collections, propagation of these plants through *in vitro* asymbiotic seed germination and tissue culture techniques, and creation of the orchid exhibition greenhouse, *Orchidarium* (fig. 1).

FIGURE 1. Fragment of the orchid display Orchidarium.

At present the NBG living collection of tropical orchids contains about 4500 plants representing approximately 170 genera and 450 natural species (not counting artificial hybrids and cultivars). Most of species of the collection (more than 70%) are native to South-East Asia, whereas the remaining 30 % are orchids from South America and Central America, with a few genera from Africa (including Madagascar). Since 1999 the whole NBG collection of tropical plants (including orchids) has the status of a National Heritage Collection of Ukraine. The list of National Heritage units, including the most important science and arts collections, is designated by relevant ministries and agencies and approved by the Cabinet of Ministers of Ukraine.

The NBG collection is taxonomically representative; it includes the taxa belonging to four out of five currently recognized subfamilies (Chase 2005, Pridgeon *et al*. 2005) of Orchidaceae (Cypripedioideae, Vanilloideae, Orchidoideae, and Epidendroideae).

The major part of the plant collection was accumulated by NBG researchers during the 1980-1990s through field trips and collecting activities in the wild in various floristic regions of Paleotropis and Neotropis, usually in close cooperation with local botanists. In addition to that, samples of plants were received as gifts from botanical gardens throughout the world (Caracas, Venezuela; Beijing, China; Warsaw, Poland; Moscow & St. Petersburg, Russia), donated by private persons, as well as purchased from well-known floricultural commercial companies (Mandai Orchids, Singapore; Vacherot & Lecoufle, France; Floriana, Brazil; Winkler's Orchids, Argentine; Saigon Orchids, Vietnam, and others).

The most valuable part of the orchid collection is represented by orchid species of the flora of Vietnam. This collection, comprising about 1/5 of the total number of species in the orchid flora of Vietnam, was developed due to scientific collaboration between NBG and the Center for Biodiversity and Development of the Institute of Tropical Biology of Vietnam. Within the framework of partnership between these institutions, five expeditions have been carried out, which resulted in new accessions to the living orchid collection.

During the recent years the orchid collection is replenished and managed taking into consideration the international priorities in the Garden's policy of collection development, as outlined in the International Agenda for Botanic Garden in Conservation (Wyse Jackson & Sutherland, 2000), CITES Orchid Checklists (Roberts *et al.*, 1995, 1997, 2001) and Global Strategy for Plant Conservation (2002). The NBG collection of tropical orchids was registered at the Administrative Organ of CITES in Ukraine (Ministry of Environment, registration No. 6939/19/1-10 of 23 June 2004).

While creating the collection, the strategic goal was to represent most widely the floristic, ecological and morphological diversity of Orchidaceae, with an emphasis on rare and vulnerable orchid species.

Botanical gardens maintaining the collections of tropical plants are responsible for their long-term persistence and sustainability, which is extremely topical at present, when there are strict limitations of CITES concerning sampling plants from natural habitats. To achieve successful acclimatization of orchid plants collected in the wild (commonly on fallen trunks and branches of dead trees), ecological requirements of each species must be met under glasshouse conditions.

Though the orchid family as a whole occupies a huge range of diverse habitats, individual species commonly demonstrate restricted distribution patterns with specific habitat preferences (Cribb, 1998, Vu Ngoc Long, 2002, Averyanov & Averyanova, 2003, Averyanov *et al.*, 2003, Pridgeon *et al.*, 2005).

Precise data on ecological requirements of many tropical orchid species remain surprisingly poorly known, and it is especially alarming if we consider an incredible rate of degradation of primary tropical forests. Thus, the best way to fill the gap in information on ecological requirements of tropical orchids is field observations in the wild.

Long-term maintenance and reproduction of live plants under glasshouse conditions with the aim of *ex situ* biodiversity conservation and subsequent repatriation are possible only on the basis of investigations of their ecological requirements, developmental biology both *in situ* and under glasshouse conditions, vegetative architecture, and anatomical and ecophysiological peculiarities.

Understanding of these peculiarities of orchids determines the adaptability of orchid plants of different ecological groups (terrestrials, epiphytes, and lithophytes)

under glasshouse conditions. On the other hand, studying of this subject is the prerequisite for development of techniques and procedures for propagation and cultivation.

The collection of orchids at the National Botanical Gardens consists of more than 450 species, all of which cannot be studied in detail, considering available resources and reasonable time limits. Because of that, for in-depth studies we identified priority groups, including such genera as *Angraecum* Bory, *Calanthe* R.Br., *Cattleya* Lindl., *Coelogyne* Lindl., *Cymbidium* Sw., *Dendrobium* Sw., *Laelia* Lindl., *Paphiopedilum* Pfitz.

While selecting the taxa, the following features were taken into account: frequency of occurrence in natural habitats, number of taxa per genus in the NBG collection, economic value (ornamental and medicinal plants), vegetative architecture, and conservation status. The preference was given to genera most widely represented in our collection, with special reference to rare orchid species of South-East Asia and South America.

Mass-propagation of orchid plants is a critical component of any long-term *ex situ* conservation program. Application of *in vitro* propagation techniques to rare tropical orchid species is, undoubtedly, a powerful tool for *ex situ* biodiversity conservation. Until recently, many tropical native orchids species from South America (*Cattleya* spp., *Laelia* spp., *Oncidium* spp.), South-East Asia (*Calanthe* spp., *Coelogyne* spp., *Dendrobium* spp., *Paphiopedilum* spp.), Africa and Madagascar (*Angraecum eburneum* Bory, *A. sesquipedale* Thouars) were propagated at the NBG through a range of asymbiotic seed germination techniques and tissue culture procedures aimed at preserving a number of individuals under artificial conditions in glasshouses of the temperate zone, and in protecting in such way these species from extinction (Cherevchenko, 1984, Buyun *et al.*, 2004, Lavrentyeva *et al.*, 2005).

Development of propagation methods for numerous species of tropical orchids in the NBG was preceded by long-term observations and dedicated studies in their reproductive biology (duration of anthesis, terms of pollination of flowers, and duration of fruit maturation).

Under glasshouse conditions, where specific pollinators are absent, artificial pollination of flowers is the only way to obtain fruits with viable seeds. For this reason, hundreds combinations of pollinations of flowers belonging to different species have been carried out in NBG greenhouses, depending on the breeding systems and quantity of samples of the species studied. As a result of these experiments, we obtained seeds of more than 100 species.

The list of natural species of *Paphiopedilum* propagated in NBG in *in vitro* culture includes up to 10 species (*P. appletonianum* (Gower) Rolfe, *P. callosum* (Rchb.f.) Stein, *P. delenatii* Guillaum., *P. insigne* (Wall. ex Lindl.) Pfitz., *P. lawrenceanum* (Rchb. f) Pfitz., *P. villosum* (Lindl.) Stein, *P. wardii* Summerh.). The urgency of protection of these species both *in situ* and *ex situ* is dictated not only by increasing demand for these plants in the world but also by their developmental biology and habitat preferences and peculiarities. Many *Paphiopedilum* species are obligate lithophytes; therefore, after the fires their populations are not restored at all (Averyanov, *et al.*, 2000). The plants propagated *in vitro* meet the huge demand for these plants, thus reducing the pressure on natural populations. *Paphiopedilum delenatii* is an excellent object to illustrate how a rare plant can be saved and propagated in cultivation for a long time (Cribb, 1998).

Undoubtedly, the use of glasshouse collections of living plants grown under artificial climate conditions in the temperate zone cannot ensure conservation of the whole genetic diversity of orchids. This way of conservation of tropical plants can be viewed rather as urgent measures because when rare species are poorly sampled only a miserable portion of their actual genetic diversity is preserved. In addition, the plant samples are often borrowed from living collections of other botanical gardens after long-term cultivation under glasshouse conditions.

Taking into account all mentioned above, the main trends of the use of fund collections for conservation of orchid diversity is to satisfy the increasing demand for plants of the natural orchid species through using seedlings and plantlets propagated *in vitro*, as well as creation of orchid exhibits.

At present the protection of biodiversity of rare tropical orchid species *ex situ* should not be limited by listing the specimens of rare and vulnerable species main-

tained in greenhouses of botanic gardens of the temperate zone climate. It requires fundamental understanding of factors controlling orchid plant development and acclimatization/adaptation in artificially created conditions using different experimental methods. These methods should be specifically designed for solving numerous theoretical and practical issues of adaptation of orchid species cultivated under glasshouse conditions. The availability of the collections of orchid species belonging to different ecological types opens wide prospects for investigating different types of life strategies, which provide survival in a wide range of ecological conditions, both in nature and in glasshouse. Finally, this will contribute to development of the most appropriate propagation methods and cultivation techniques under glasshouse conditions.

The original results of studies of seed coat patterns is an example of such investigations undertaken in NBG for elucidating structural morphological adaptations of orchids to different ecological niches. Seed testa sculpture patterns of more than 140 tropical orchid species belonging to 70 genera were investigated in NBG using scanning electron microscope (SEM) (Buyun, 2006). Actuality of this investigation can be explained by the fact that seed coat sculpture, as well as external surface sculpture of any plant organ directly exposed to the environment, can bring important information reflecting the pathways of morphological adaptation of orchid plants to specific environmental conditions (Kurzweil, 1993, Thompson *et al.*, 2001). Thus, ecological considerations of differences between seed coat sculpture of studied orchids (epiphytes, terrestrials, and lithophytes) highlighted by SEM are useful both for enhancing *in vitro* seed culture as a means of promoting conservation of tropical orchids *ex situ* and for elaborating appropriate techniques for their cultivation under glasshouse conditions.

To summarize, the main aspects of tropical orchid investigations in the NBG cover the following fields: (1) studies of developmental biology of orchids under glasshouse conditions (with special reference to reproductive biology of epiphytes and lithophytes as the most vulnerable groups); (2) investigation of structural adaptations of orchids to survival under a wide range of different habitats; (3) development of *in vitro* orchid propagation methods and cultivation techniques.

Beside this, the exhibition greenhouse *Orchidarium*, which was opened for public in 2005, can be considered as a source of material for scientific investigations, a wide range of educational programs, as well as an efficient tool in raising public awareness in issues related to conservation of rare tropical orchid species suffering from over-collecting and continuous loss of their natural habitats.

Two international conferences on biology and conservation of tropical and native orchids were held at NBG in 1983 and 1999, which emphasize the role of NBG as a leading Ukrainian center of *ex situ* conservation of tropical orchids.

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LITERATURE CITED

Averyanov, L.V., Nguyen Tien Hiep, Phan Ke Loc & A.L. Averyanova. 2000. Preliminary orchid checklist of Cao Bang Province. Lindleyana. 15 (3): 130-164.

Averyanov, L.V. & A.L. Averyanova. 2003. Update checklist of the orchids of Vietnam. Hanoi: Vietnam National University Publishing House. 102 p.

Averyanov, L., P. Cribb, Phan Ke Lock & Nguyen Tien Hiep. 2003. Slipper Orchids of Vietnam. Portland, Oregon: Timber Press. 308 p.

Buyun, L.I. 2006. Seed coat features of tropical orchids (*Orchidaceae* Juss.). Proceedings of the 12th Congress of Ukrainian Botanical Society. 408 (in Ukrainian).

Buyun, L., A. Lavrentyeva, L. Kovalska & R. Ivannikov. 2004. *In vitro* germination of some rare tropical orchids. Acta Universitatis Latviensis. Biologia. 676: 159-162.

Chase, M.W. 2005. Classification of *Orchidaceae* in the age of DNA data. Curtis's Botanical Magazine. 22 (1): 2–7.

Cherevchenko, T.M. 1984. Tropical orchids. Morphological study and cultivation under greenhouse conditions. Abstract of Doctoral Thesis. Kiev. 44 p (in Russian).

Cribb, P. 1998. The genus *Paphiopedilum*. Kota Kinabalu: Natural History Publications. 1998. - 427 p.

Cribb, P, D. Roberts & J. Hermans. 2005. Distribution, ecology, and threat to selected Madagascan Orchids. Selbyana. 26 (1, 2): 125-135.

Dunkan, M., A. Pritchard & F. Coates. 2005. Major threats to endangered orchids of Victoria, Australia. Selbyana. 26 (1,2): 189-195.

Global Strategy for Plant Conservation. 2002. Approved in Decision VI/9 of the Conference of the Parties (COP) to

the Convention on the Biological Diversity, on 19 April. The Hague.

Grodzynsky, D.M., Sheliag-Sosonko, R. Yu., T.M. Cherevchenko, G. Yemelyanov, V.G. Sobko & A.P. Lebeda. 2001. Problems of conservation and renewal of biodiversity in Ukraine. Kyiv: Academperiodyka. 106 p. (in Ukrainian).

Kurzweil, H. 1993. Seed morphology in southern African Orchidoideae (Orchidaceae). Plant. Syst. E. 185 : 229-247.

Lavrentyeva, A.M., L.I. Buyun & L.A. Kovalska. 2005. Seed propagation of *Dendrobium draconis* Rchb.f. (*Orchidaceae* Juss.) *in vitro* culture. Bulletin of Kyiv National University. 9: 36-37 (in Ukrainian).

Pridgeon, A.M., P.J. Cribb, M.W. Chase & F. Rasmussen (eds.). 2005. Genera Orchidacearum. Volume 4: Epidendroideae (Part 1). Oxford University Press, Oxford. 672 p.

Roberts J.A., C.R. Beale, J.C. Benseler, H.N. McGough & D.C. Zappi. 1995. CITES Orchid Checklist. Vol. 1. Royal Botanic Gardens, Kew. 136 p.

Roberts J.A., C.R. Allman, C.R. Beale, R.W. Butter, K.R. Crook & H.N. McGough. 1997. CITES Orchid Checklist. Vol. 2. Royal Botanic Gardens, Kew. 300 p.

Roberts J.A., S. Anuku. J. Burdon. P. Mathew, H.N. McGough & A.D. Newman. 2001. CITES Orchid Checklist. Vol. 3. Royal Botanic Gardens, Kew. 232 p.

Thompson, D. E., T.J. Edwards & J. van Staden. 2001. In vitro germination of several South African summer rainfall *Disa* (Orchidaceae) species: is seed testa structure a function of habitat and a determinant of germinability? Syst. Geogr. Pl. 71: 597-606.

Vu Ngoc Long. 2002. Genus *Eria* Lindl (Orchidaceae Juss.) in the flora of Vietnam: morphological evolution and taxonomy. Abstract of PhD Thesis. Kiev. 22 p.

Wyse Jackson, P.S. & L.A. Sutherland. 2000. International Agenda for Botanic Gardens in Conservation. Botanic Gardens Conservation International, U.K. 56 p.

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Lyudmyla I. Buyun graduated from Kyiv National University in 1982. She received her PhD degree in biology from the National Botanic Garden, Kiev, in 1987, for a study of developmental biology of deciduous *Calanthe* species under greenhouse conditions. At present Lyudmyla is Senior Researcher and the orchid collection curator at the National Botanical Garden. She is particularly interested in reproductive biology as well as structural adaptations of orchids of different ecological groups.

Lyudmyla A. Kovalska graduated from Kyiv National University. She has been growing orchids for more than 30 years. She received her PhD degree in biology in 1993 for a study of developmental biology and in vitro propagation of *Dendrobium phalaenopsis* as a horticulturally valuable plants. At present she is the orchid collection curator; her particular interests are vegetative architecture and morphogenesis of orchids. Lyudmyla is also a curator of the fern collection.

Vu Ngoc Long graduated from Hanoi University in 1978. He completed his postgraduate course at the V.L. Komarov Botanical Institute (St. Petersburg, Russia). He received his PhD degree in biology from the National Botanic Garden of the National Academy of Sciences of Ukraine. He is particularly interested in taxonomy and morphological evolution of the genus *Eria* Lindl. in the flora of Vietnam. At present he serves as Director of the Center for Biodiversity and Development and Vice-Director of the Institute of Tropical Biology in Vietnam. He is also involved in several conservational projects in South-East Asia.

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EL SISTEMA LANKESTER

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En muchas de las charlas, el ponente presenta su charla en inglés para favorecer a los visitantes extranjeros. En este caso, creo que el material que quiero presentar es de más interés para los visitantes de América Tropical que para los de habla inglesa. En mi experiencia, todos los jardines botánicos, tal vez con excepción de Longwood, hallan que no tienen los recursos suficientes para para hacer lo que deben hacer. En la realidad, la mayoría de los jardines botánicos en los Estados Unidos y Europa tienen más apoyo y más recursos que la mayoría de los jardines botánicos en los trópicos. Para que un jardín sea un jardín botánico, creo que el elemento más crítico es un sistema de datos. En el Jardín Botánico Lankester (JBL) ya tenemos un sistema que funciona con poca plata, y creo que les puede ser de interés. Normalmente, lo llamamos "el sistema Lankester," pero igualmente lo podríamos llamar el sistema Pupulin.

En muchos de los jardines botánicos de zonas templadas, hay una oficina de "adquisición." Todas las plantas que entran al jardín tienen que recibir un número en esta oficina, lo cual implica al menos una persona a tiempo completo. En nuestro caso, tenemos pocas personas a tiempo completo, pero sí tenemos una serie de estudiantes que trabajan unas horas cada semana. Tenemos la intención de dar un número, más bien de "acceso" que de adquisición, a cada planta que se cultiva en nuestros invernaderos. Para que este sistema funcione, usamos también números de colecta de cada estudiante y empleado que colecte plantas, para que haya un sistema que asocie cada planta con sus datos de origen, hasta que la planta tenga su número de acceso del jardín.

En el caso de material conservado, el material prensado en el campo recibe su número de colecta del colector, aún cuando una parte de la misma planta puede ser cultivada en el invernadero (con el mismo número). Por ahora, no mantenemos una colección permanente de material prensado, pues bajo nuestras condiciones de humedad, ninguna colección de material prensado puede ser permanente. Normalmente depositamos material en el Herbario de la Universidad de Costa Rica (USJ) o en el Museo Nacional (CR). La humedad no interfiere con una colección de material en alcohol, y en el JBL mantenemos una colección muy útil de material en alcohol. A mi modo de ver, lo ideal sería agrupar el material en un orden sistemático o filogenetico, pero en la práctica usamos un sistema por tamaño de frasco - pequeño, mediano, grande o largo, y hay una base de datos, por lo cual uno puede accesar por género o especie.

Ahora usamos unas etiquetas en las cuales uno puede marcar si hemos prensado material, si hay material en líquido, o si hay material para ADN (en silica), si hay foto o dibujo de la planta.

Franco ha inventado un sistema en que se escanea la planta, la flor, o partes de la flor, y uno puede hacer un buen dibujo de estos escaneos. Por cierto, hay que tener cuidado de no perder a algunos detalles superficiales, que pueden desaparecer o disminuir cuando se aplastan las partes florales.

Robert L. Dressler obtuvo su doctorado en la Universidad de Harvard y laboró con el Jardín Botánico de Missouri y el Instituto Smithsoniano para la Investigación Tropical. Es investigador asociado al Herbario de la Universidad de Florida, el Jardín Botánico de Missouri y el Jardín Botánico Mary Selby. Es autor de centenares de artículos científicos y de reconocidos libros sobre historia natural, filogenia y clasificación de las orquídeas. Su principal interés se centra en la fiologenia y taxonomía de la subtribu Sobralinae. Actualmente labora para el Jardín Botánico Lankester de la Universidad de Costa Rica donde se desempeña como Coordinador de Investigación.

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HOW DOES HYBRIDIZATION INFLUENCE THE DECISION MAKING PROCESS IN CONSERVATION? THE GENUS *ORCHIS* (ORCHIDACEAE) AS A CASE HISTORY

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KEY WORDS: AFLP, hybridization, introgression, ITS rDNA, *Orchis*, plastid microsatellites

Hybridization is a fundamental process in biology and can lead to new evolutionary lineages. However, if the parental taxa involved are rare, difficult decisions may have to be made regarding the conservation of the biological process versus the conservation of the parental taxa. The genus *Orchis* in Europe is a good example of a group of species in which these types of questions arise as several of the species hybridize where they co-occur. The example used here relates to *O. militaris*, *O. purpurea* and *O. simia* in the anthropomorphic group (so called because the labellum has lobes thought to resemble arms and legs). All three species are widespread in Europe, although they are rare in large parts of their ranges, and they have substantial areas of overlap in distribution. All three are rare in Britain, occurring predominantly in south east England. *Orchis militaris* and *O. simia* and are only known from two and three natural sites in England, respectively. *Orchis purpurea* is less rare, but is still geographically localized.

Morphological inspection of plants provides convincing evidence of widespread hybridization in different parts of the range of these species. In addition, preliminary genetic data indicate that there is some introgression of genetic markers in populations that appear on a morphological basis to be representatives of one species rather than hybrids (Fay & Krauss, 2003). Finally, we know of a number of populations in which hybridization is currently occurring, including one in England where *O. purpurea* has recently appeared at one *O. simia* site. The first hybrids flowered in 2006.

To investigate patterns of hybridization and introgression in these species further, we compiled data sets for three types of markers: sequences of the nuclear ribosomal spacer regions (nrITS), plastid microsatellites, and amplified fragment length polymorphisms (AFLP). These markers have now been applied to samples from populations of all three species and some putative hybrids, and we are currently expanding our sample size to include individuals from a wider geographical range.

The new ITS sequences were added to a subset of those in the matrix of Pridgeon *et al*. (1997), and phylogenetic analyses were carried out. The plastid microsatellite markers were developed for use with a range of *Orchis* spp., including the anthropomorphic group and their relative *O. mascula*. Five regions showing length variation within and between species were identified (as described in Fay and Krauss, 2003), and primers were designed to allow the amplification of short fragments (90-230 base pairs) that contained the length-variable regions. Then differences between individuals were detected by assessing the length of the amplified fragments using an automated sequencer. Together the alleles for these five regions provide a haplotype for each individual and a minimum spanning tree was constructed from the haplotypes. AFLP were carried out using a modified version of the protocol of Vos *et al*. (1995). The resulting 0/1 matrix was analysed using principal coordinates analysis and neighbor joining.

All three data types can provide information relating to hybridization. Plastid DNA provide informa-

tion relating to the maternal parent of any hybrids. ITS rDNA is part of the nuclear genome and it is inherited biparentally, but hybrids soon begin a process of gene conversion and lose one of the two parental copies that they initially possessed. For fairly recently synthesized hybrids, both parental ITS alleles are present, but for older hybrids only one of these alleles (often that from the maternal parent) normally remains. AFLP have been widely used in studies of hybridization, because hybrids show additivity between the profiles found in the parental species and the hybrids consequently fall in an intermediate position between the parents in ordination plots (e.g., Fay *et al*., 2003).

Four different ITS sequence types (each with minor variants) were identified, one each for *O. militaris* and *O. simia* and two for *O. purpurea*. In addition, some individuals of *O. purpurea* and *O. simia* had the ITS copy type of *O. militaris*. Individuals of intermediate morphology (presumed recent hybrids) had ITS copies from both putative parents. Plastid microsatellites did not provide such a clear division, but there were two major clusters identified with these markers, consisting predominantly of *O. militaris* and *O. simia*, respectively, but each also containing some individuals of *O. purpurea*. Most hybrids and some individuals of *O. simia* fell in the *O. militaris* cluster. AFLP identified distinct clusters for the three species, and the cluster for *O. purpurea* was divided into two subclusters. Hybrids fell in intermediate positions with AFLP as expected.

These data indicate that *O. militaris* and *O. simia* are good species that sometimes hybridize, leading to occasional plastid and ITS capture. In all individuals showing evidence of introgression and hybrids studied, plastid DNA indicated that *O. militaris* was the female parent. While our data set is still somewhat limited, it thus appears that hybridization occurs predominantly in one direction in these species. The situation with *O. purpurea* was not, however, so clear. With ITS and with AFLP, there is a suggestion that this species as currently circumscribed includes two genetic entities. In contrast, we have still to identify a plastid type characteristic of this species, if one exists. Many English populations always believed to be *O. purpurea* contain individuals with the ITS type normally associated with *O. militaris*, and there is a possibility that this is the situation elsewhere in Europe. The situation is clearly complicated, and to resolve the status of *O. purpurea*, we will need to improve our sample size for both molecular and morphological studies.

How does all this relate to conservation? Hybridization between species in this group is clearly part of an ongoing process, and the species involved appear to be able to maintain pure populations despite this process. At the edge of the species range, however, there is a risk that the peripheral populations of pure individuals may be lost due to hybridization. In England, *O. purpurea* has expanded its range in recent years, possibly due the warmer summers, and it has now formed a population sympatric with one of the two remaining natural populations for *O. simia*. To the dismay of the managers of the nature reserve, several plants flowering for the first time in 2006 were morphologically intermediate and genetic evidence has shown them to be hybrids with *O. purpurea* as the female parent. As both species are rare in England, this situation has received widespread attention, and we are currently discussing the situation with the managers of the site and with the national conservation agency, Natural England. Various suggestions have been made, ranging from letting nature take its course to digging up the hybrids and the individuals of *O. purpurea* to protect the remaining pure individuals of *O. simia*. No decision has yet been made, but this should be done before the plants flower again in the 2007 season.

This and other examples of hybridization in European orchids will be used to illustrate the decision-making processes involved and the relative merits of conservation of named species versus the conservation of process.

LITERATURE CITED

Fay, M.F., R.S. Cowan & D.A. Simpson. 2003. Hybridisation between *Schoenoplectus tabernaemontani* and *S. triqueter* (Cyperaceae) species in the British Isles. Watsonia 24 : 433-442.

Fay, M.F. & S.L. Krauss. 2003. Orchid conservation genetics in the molecular age. Pp. 91-112 *in*: K.W.

Dixon, S.P. Kell, R.L. Barrett & P.J. Cribb (eds.). Orchid conservation. Natural History Publications, Kota Kinabalu, Sabah.

Pridgeon, A.M., R.M. Bateman, A.V. Cox, J.R. Hapeman & M.W. Chase. 1997. Phylogenetics of subtribe Orchidinae (Orchidoideae, Orchidaceae) based on nuclear ITS sequences. 1. Intergeneric relationships and polyphyly of *Orchis sensu lata*. Lindleyana 12 : 89-109.

Vos, P., R. Hogers, M. Bleeker, M. Rijans, T. Van de Lee, M. Hornes, A. Frijters, J. Pot, M. Kuiper & M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23 : 4407-4414.

Mike Fay received his undergraduate degree in Genetics and Plant Breeding from the University College of Wales, Aberystwyth. His graduate studies were on *Trifolium*, and he received his Ph.D. from the University of Wales in 1989. He joined the staff of the Royal Botanic Gardens, Kew, in 1986 as Head of Micropropagation, giving him the opportunity to become involved professionally with orchids. In 1995, he established a conservation genetics program at Kew, and since 2002 he has been Head of Genetics. Many projects past and present are on orchids. He recently became Chair of the Orchid Specialist Group of IUCN.

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TROPICAL ORCHIDS IN THE NORTH, MONTRÉAL BOTANICAL GARDEN, QUÉBEC, CANADA

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KEY WORDS: orchid collection, history, conservation, Botanical Garden

The Montréal Botanical Garden was founded, in 1931, by the remarkable botanist and scientist, catholic brother Marie Victorin. Since its foundation the garden has three missions : conservation, education and research. Certainly 76 years old is still young for a botanical garden, but it has accomplished a maturity and dynamism that is admired by many. The tropical collections are presented to the public by family and theme in ten public greenhouses. Behind the scene, more than 40 greenhouses serve for conservation of our collections, for production and for research. Situated on 75 hectares, 27 thematic gardens present the different collections while three gardens have a cultural vocation: the Chinese Garden, the Japanese Garden and the American Indian Garden. A total of 22,000 taxons are presented in our collections.

Considering its size, the richness of its collections, the number of employees and the number of visitors (1,000,000/ year), the Montréal Botanical Garden ranks as one of the five largest in the world.

Henry Teuscher, co-founder of the Montréal Botanical Garden, designed the garden and supervised its construction. He was appointed as curator in 1942, a position he occupied until his retirement in 1962. During this 20 year period he established the basis of the collections, particularly the orchid collection, for which he had a real passion. He wrote hundreds of articles for the *American Orchid Society Bulletin* under the title of 'Collector's Item', articles which were very much appreciated by collectors of species. Mr. Teuscher became more and more interested in taxonomy and collaborated with Leslie A. Garay, taxonomist botanist at Oakes Ames Herbarium at Harvard University in Boston, MA (U.S.A) Mr. Garay identified some new species from samples from the Botanical Garden collection and he named the genus *Teuscheria* in honour of Mr. Teuscher. Mr. Teuscher's contribution to the knowledge of orchids was the first and the most important in Eastern Canada.

Early in his career, in the years of 1940-1950, Mr. Teuscher established contacts with different botanical gardens recognized for their orchid collections and he solicited donations and exchanges. As a result of his efforts, the New York Botanical Gardens (Bronx, NYC, U.S.A), the Palmentengarten of Frankfort (Germany) and the National Botanical Garden of Dublin (Ireland) as well as many others contributed to the formation of the nucleus of our orchid collection. From 1945 to 1975 the development of our collection focused on South American species sent by two orchid hunters, J. Strobel of Cuenca, Ecuador and C.K. Horich of San Jose, Costa Rica. The specimens were often identified only by genus, sometimes simply as 'orchid number…'. However, they included information concerning habitat, altitude, color of flowers, collection site etc. This information facilitated both cultivation and identification of plants.

Pierre Bourque, director of the garden from 1980-1994, gave new life to the development of the collection and promoted its visibility by giving the collection its own public greenhouse. He was fascinated by orchids, their capacity to adapt and by their power of attraction. During his many overseas trips : Costa Rica, Ecuador, Hong Kong etc, he always had the orchid collection in mind and he brought back numerous species.

Also, we have had and continue to receive occasional donations from collectors who wish to assure the perpetuity of their collections by leaving their orchids to the Montréal Botanical Garden. These

donations as well as exchanges with other institutions and with private collectors allow us to continue to enrich our collection. However, presently the majority of our acquisitions are plants we purchase from specialized producers who now offer a large variety of species.

For all of these reasons, our collection currently consists of 60% South American species, 30% Asiatic species and 10% African species. The Montréal collection contains 270 genus, 1,440 species and 1,998 taxons, for a grand total of approximately 5,000 specimens (because we keep a minimum of two plants per taxon). We wish to maintain our historical emphasis on South America and to continue to add to the number of South American species in our collection.

To manage our collection, we use a data base named BGbase (Botanical Garden base) which permits us to keep information on each plant - collection site, geographic distribution, synonym, changes of nomenclature, etc. - enabling us to follow the evolution of each plant in our collection. With this data we can compile various detailed reports of the number of genus, number of species, number of IUCN plants, etc. This information is quickly and easily available to all horticultural personnel of the Botanical Garden.
Our orchids are cultivated in three computer controlled greenhouses. The parameters of temperature, relative humidity, light levels and ventilation are precisely controlled, resulting in the exact climate necessary for their growth. The collection is maintained in four greenhouses, one of which is our 217 square meter public greenhouse where our visitors can appreciate orchids in flower 12 months of the year with new plants added each week.

Visitors have the choice of guided tours, where they can learn the basics of the orchid family, or they may visit the collection on their own. The flowering is particularly abundant between January and June. The three other greenhouses are production greenhouses. We have a 285 square meter hot greenhouse, a 354 square meter intermediate greenhouse, and a smaller 112 square meter cold greenhouse. During the last several years, we have experimented with mixtures of various materials : peat moss, sphagnum moss, rock wool, clay pellets, coconut bark, and the synthetic material epiweb. We test these various materials on different genus with the goal of finding the best mixtures which will perform well for our entire collection. We evaluate our mixtures over a two to three year period. During this time, we observe plant growth, measure pH and salinity levels and analyse soil and leaf samples. We also improved our fertilization methods. We work with ferti-irrigation of 100-150 ppm, using low phosphorous fertilizers, we use mainly nitrates, and we also add magnesium in the form of Epsom salt at each watering. Finally, one time per month, we add calcium and iron. We have observed an improvement in the growth and flowering of our plants and the number of CCM and CCE awarded for our plants in the past several years attest to this success. The Garden also has a laboratory where we practice in vitro propagation of rare, difficult or virus infected plants and the follow up of plants received in flasks.

At the Montreal Botanical Garden, we play an important de-facto role in conservation, considering the fact we have a collection of more than 5000 specimens, mainly species, many of which appear on the IUCN Red List. We plan to continue acquiring rare, vulnerable, or endangered species. We continue our mission of educating and raising public awareness through guided tours of our public orchid greenhouse, courses at the Botanical Garden school, and conferences offered to various horticultural groups.
In the future we would like to directly participate in ex-situ and/or in-situ conservation projects. We believe we have the knowledge, experience and facilities necessary to be valuable partners in orchid conservation efforts.

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FURTHER READINGS

Argo, B. 2003. Understanding pH management and plant nutrition. J. Intern. Phalaenopsis Alliance 12(4), 13(1-3).
Bouchard, A. 1998. Le Jardin botanique de Montréal: esquisse d'une histoire. Edition Fides, 111p.
Bulletin de la société d'animation du Jardin et de l'Institut botaniques 10(4): novembre-décembre.

Quatre-Temps. 2006. Le Jardin botanique fête ses 75 ans! Quatre-Temps, La revue du Jardin botanique de Montréal 30(2-3).

Quatre-Temps. 1993. Les orchidées. Quatre-Temps, La revue du Jardin botanique de Montréal 17(1).

Stewart, J. 1995. Manual of orchids. Timber Press, Portland, 388 p.

Teuscher, H. 1940. Programme d'un jardin botanique idéal. Jardin botanique de Montréal, Montréal, 33 p.

Yin-Tang W. & L.G. Lori. 1994. Medium and fertilizer affect the performance of *Phalaenopsis* orchids during two flowering cycles, HortScience 29(4): 269-271.

Yin-Tung W. 1995. Medium and fertilization affect performance of potted *Dendrobium* and *Phalaenopsis*. Hort Techn. July- September.

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THE CONSERVATION DILEMMA

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RESUMEN: Aunque el Estado de Florida (Estados Unidos) ha adoptado las directrices de la UICN en cuanto a la aplicación de los criterios de conservación relacionados a su fauna, existe un vacío en términos de que ciertas especies pueden ser protegidas a pesar de no encontrarse en la lista de especies amenazadas y recibir algún nivel de protección. El Parque Nacional Everglades (Florida) es un hábitat crítico para un número de especies raras y otras varias docenas enlistadas como En Peligro o Amenazadas. Sin embargo, un dilema es la interacción entre especies. En este caso, la mosca endémica *Melanagromyza miamensis* oviposita sus huevos en la inflorescencia de la orquídea *Trichocentrum undulatum.* En ella, las larvas se desarrollan generalmente matando al tallo. Este es un ejemplo de la interacción entre dos especies, una ampliamente distribuida pero localmente incluida bajo la categoría de Críticamente Amenazada (CR) (*T. undulatum)* y la especie endémica (*M. miamensis*). De esta manera, se utiliza este ejemplo para demostrar la complejidad en el desarrollo de un plan de manejo.

KEY WORDS: conservation, Everglades National Park, *Melanagromyza miamensis, Trichocentrum undulatum*

Introduction

The historic Everglades are a vast wetlands of international significance. South Florida is one of the most biologically diverse regions in North America, harboring over 1,400 species of native plants. Everglades National Park, the largest subtropical wilderness in the United States, constitutes a single, biotic engine that drives the systems that support all life in South Florida. The area boasts many rare and endangered species. The commingling of tropical and temperate plants, alligators and crocodiles, freshwater and saltwater swamps is found nowhere else on Earth. It has been designated an International Biosphere Reserve, a World Heritage Site, and a Wetland of International Importance, in recognition of its significance to all the people of the world. As such, this unique ecosystem has been the focus of the largest hydrologic restoration program ever attempted. The Comprehensive Everglades Restoration Plan (CERP) will provide multiple benefits to the south Florida ecosystem. With the implementation of the plan, improvements will be made by restoring natural flows of water, water quality and hydroperiods, improving the health of the south Florida ecosystem including the Everglades and Biscayne National Park, improving hydrologic conditions which will result in Lake Okeechobee once again becoming a healthy lake, and improving health of native flora and fauna populations including the threatened and endangered species.

HISTORY. Water is the lifeblood of the South Florida ecosystem. The Central and Southern Florida (C&SF) Project was first authorized by Congress in 1948 as a multi-purpose project intended to provide flood control; water supply for municipal, industrial, and agricultural uses; prevention of saltwater intrusion; a water supply for Everglades National Park; and protection of fish and wildlife resources. The primary system included about 1,000 miles of levees, 720 miles of canals, and almost 200 water control structures. Although the U.S. Army Corps of Engineers had good intentions, the results have been disastrous. Not only does approximately 70 percent less water flows through the ecosystem today as compared to the historic Everglades, but the quality of the water that does enter the ecosystem has been seriously degraded. It does not follow the tim-

ing and duration of the historical Everglades, nor can water move freely throughout the entire system. The whole South Florida ecosystem has suffered. The health of Lake Okeechobee, the second largest freshwater lake wholly in the United States, and an important home to fish and wildlife, is seriously threatened. A number of plants and animals that live in South Florida and the Everglades are in danger of becoming extinct because their habitat has been damaged, reduced, or eliminated. Clean water is not available to the estuaries and bays that are critical nurseries and homes to many fish and wildlife. Nor is there enough water for the humans. Water shortages and water restrictions are now a way of life in South Florida.

THE DILEMMA. Conflicting goals of habitat restoration, flood control, water storage, and transportation compete with prioritization of resource allocation. Within habitat restoration, priorities must be set to determine which species need intervention and what actions should be taken. The U.S. Army Corps of Engineers, in partnership with the South Florida Water Management District, has developed a Comprehensive Everglades Restoration Plan (CERP) to save the Everglades. Two aspects of the plan are considered here: waterflow and habitat restoration.

From a conservation standpoint restoring the southward flow of water is simply a matter of removing the obstacles to southward waterflow (dikes and roads) and plugging east-west diversions (canals and waterways). However, the dikes retain water for agricultural and municipal storage in the dry season and flood control in the wet season. The agricultural muck areas south of Lake Okeechobee were historically subject to sheet flow in the rainy season. Two major east-west roads in South Florida impede waterflow: I-75 and US-41. Numerous canals in developed areas of South Florida drain residential areas by cutting through the coastal ridge, diverting waterflow into the ocean. The Okeechobee Waterway connects the east and west coasts of Florida by connecting Lake Okeechobee to the Caloosahatchee River and the St. Lucie Canal, which connects Stuart, Florida, to Lake Okeechobee. The effects of these man-made changes have caused significant alterations in the timing (excess wet season flows, insufficient dry season flows), distribution, quality, and volume of freshwater entering the estuaries. Excess water during rainy seasons produces low salinity levels that are unable to support marine aquatic life, while severe low flows during the dry season causes salinity levels to spike. The goal of CERP is to redirect fresh water to areas that need it most.

Saving the Everglades

The Institute for Regional Conservation (IRC), based in Miami, Florida, is dedicated to the protection, restoration, and long-term management of biodiversity on a regional basis. Their work is premised on an innovative idea of conservation that seeks to protect and restore viable populations of all plant and animal species within a region. Unfortunately, habitat destruction, collecting, hydrological modifications, fire suppression, and other human activities have heavily disturbed, if not critically imperiled, many of South Florida's ecosystems, thus threatening many native plant species. There is an alarming loss of species. Over 100 species of native plants (8%) are apparently extirpated in the region. Another 244 species (17%) are Critically Imperiled, using Natural Heritage Program criteria. Epiphytes, including rare tropical orchids, ferns, and bromeliads, are more likely to be extirpated or critically imperiled than terrestrial plants. Most apparent extirpations have occurred in the last fifty years, which coincides with the C&SF Project. Fortunately, only one endemic South Florida species, the Narrowleaf Hoarypea (*Tephrosia angustissima* Shuttlew. ex Chapm. var. *angustissima* [Gann et al. 2002]), is presumed to be extinct.

INITIAL MANAGEMENT PLAN. Conservation management plans are regional strategies that provide an overview of conservation issues and give direction for the management of public conservation land and waters, as well as of species that are threatened. The strategies are a guide for both conservation managers and the public, indicating what managers intend to do, how priorities will be set, and how managers can respond to requests to use the natural resources they manage: in other words, how to reconcile conservation of biological resources with their sustainable use.

The IRC is dedicated to long-term management of biodiversity throughout South Florida. Their plan is based on the following methods: collecting baseline

scientific data; assessing, planning and providing technical support for conservation; designing and implementing ecological restoration projects and long-term management programs; monitoring the effects of conservation projects on rare species and ecosystems and assessing needs for adaptive management; providing public education and publishing the results of their work online and in technical and popular journals; and nurturing a conservation aware community. The specific activities include

- conducting floristic inventories on conservation lands without plant data,
- conducting plant surveys on accessible private lands,
- mapping and monitoring rare plants,
- acquiring sites with populations of critically imperiled plants,
- developing conservation agreements with private landowners,
- stopping avoidable losses of rare plant populations in conservation areas,
- preventing poaching,
- controlling exotic pest plants and feral animals,
- restoring key habitats for rare plants in South Florida,
- restoring viable populations of critically imperiled plants,
- improving funding for rare plant conservation and restoration,
- developing and managing off-site collections of rare plants,
- educating the public and policy makers about the importance of native plants and rare plant conservation.

Marie Selby Botanical Gardens' Collaboration

The involvement of the scientists at Marie Selby Botanical Gardens (MSBG) with the Everglades restoration includes surveying populations, developing and managing *ex situ* collections of critically imperiled plants, and propagating germplasm for reintroduction. Presently, MSBG is propagating three imperiled ferns: *Pecluma plumula* (Humb. & Bonpl. ex Willd.) M.G.Price, the plumed rockcap fern; *Adiantum melanoleucum* Willd., the fragrant maidenhair fern; *Thelypteris reticulata* (L.) Proctor, the lattice-vein fern; and two orchids: *Brassia caudata* (L) Lindl., the Spider orchid (a Jamaican plant); and *Oncidium ensatum* Lindl., the Florida dancing-lady orchid. The fern spores and orchid seed have germinated. The fern spores were sown in pasteurized potting mix and the orchid seeds were germinated asymbiotically in-vitro.

The main concern about the reintroduction of a plant that has been extirpated from South Florida is the source of genetic material to be used for restoration. If the plant is a tropical species at the northern limit of its range, this can be a major problem. This is the case for many tropical ferns and orchids that have been extirpated from South Florida. These tropical species almost certainly arrived in South Florida from the Bahamas and Cuba; thus Bahamian or Cuban germplasm would have to be considered as appropriate sources of propagules. The *Brassia caudata*, being propagated by MSBG is a plant of Jamaican origin that has the same characteristics as the extirpated Florida species.

DATA DEFICIENT SPECIES. In the 1920s, G. Moznette collected a specimen of an unknown fly in Dade County, Florida. The specimen was deposited at National Museum of Natural History (USNM), Washington DC. Moznette labeled the specimen "orchid - larva destroys bloom." The specimen remained unidentified until 1973 when Spencer described it as a new species of Agromyzidae. Externally the fly closely resembles *Melanagromyza*

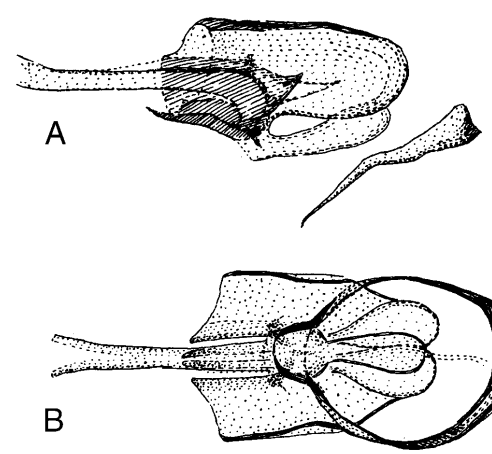

FIGURE 1. Distinct male genitalia (aedeagus) of *Melanagromyza miamensis* (Spencer 1973). **A**. side view. **B**. ventral view.

FIGURE 2. Dying buttonwood (*Conocarpus erectus)* forest converting to a saltwort marsh (*Batis maritima*). Photo by Wesley Higgins.

FIGURE 3. Site where *Melanagromyza miamensis* oviposited its eggs into the *Trichocentrum undulatum* stalk. Photo by Bruce Holst.

FIGURE 4. When ovipositing occurs below the 5th node, it usually kills the *Trichocentrum undulatum* inflorescence. Photo by Wesley Higgins.

floridensis Spencer; however, the male genitalia are entirely distinct (Fig. 1). Spencer proposed that the fly was a seed feeder and may feed on other epiphytic orchids in South Florida. The fly, possibly endemic to the Flamingo area of Everglades National Park, is not listed in USFWS Threatened and Endangered Species System (TESS). Using IUCN criteria this species is data deficient.

CRITICALLY IMPERILED SPECIES. This Critically Imperiled species selected for *ex situ* propagation is *Trichocentrum undulatum* (Sw.) Ackerman & M.W.Chase. Only one known population occurs in Everglades National Park near Flamingo. This habitat is a coastal berm forest of *Conocarpus erectus* L. (Buttonwood), where *T. undulatum* grows epiphytically. Historically, this species was also found in rockland hammocks further to the north. *Trichocentrum undulatum* is native to South Florida and the West Indies. Perhaps fewer than 500 plants exist in Everglades National Park today (R. Hammer in Gann *et al*., 2002). The major threats are habitat destruction (exotic pest plant invasions & sea-level rise), reproduction interruption, and poaching. IRC has proposed to reestablish a viable population of *T. undulatum* in the Long Pine Key area, where it historically occurred, in case that the Flamingo population is lost due to sea-level rise.

FOREST DECLINE. Although Buttonwood is salt tolerant and thrives in soils that are acidic to alkaline, clayey to sandy, and dry to wet, there is generalized forest decline in the coastal berm site where *Trichocentrum undulatum* occurs. The area is converting to a saltwort marsh of *Batis maritima* L. as the trees die (Fig. 2). This is presumably caused by a combination of increased salinity due to a disruption of fresh water flow and sea level rise. A site survey reveals that as the buttonwood trees lose their canopy, the orchids are exposed to excessive sunlight and die.

SPECIES INTERACTION. Another factor threatening the *Trichocentrum undulatum* population is disruption of the orchid reproduction cycle by an insect. Rick and Jean Seavey, naturalists from Miami-Dade County, have reported an interesting fly/orchid association (2006). The fly, *Melanagromyza miamensis* Spencer, oviposites its eggs into the stalk (Fig. 3) where the lar-

FIGURE 5. *Trichocentrum undulatum* plant generally develops side branches which are also attacked by the fly. Photo by Bruce Holst.

vae develop, later to emerge as adult flies. If ovipositing occurs below the 5th node, it usually kills the inflorescence (Fig. 4). If entered above 5th node, the plant generally develops side branches (Fig. 5) on the stalk, which are also attacked by the fly. This leads to either no flowers or an emaciated inflorescence. In 1998, only two plants flowered of 200 the Seaveys monitored. In 2006 the *Trichocentrum undulatum* population was surveyed and only one seed capsule (Fig. 6), the result of hand pollination, was found in the population. Despite the negative factors, recruits (seedlings and young plants) can be found in the healthy areas of the buttonwood forest (Fig. 7). Several specimens of *M. miamensis* Spencer were collected in Everglades National Park, near Homestead (Dade County; E97-000732; Ron Clouse, park employee; 28 February 1997). These specimens are deposited in the Florida State Collection of Arthropods (Fig. 8). The insect is very rare as this is only the second reported collection. The fly is possibly endemic to the Flamingo area of Everglades National Park.

POACHING. Public education is the primary effort to stop poaching, but enforcement is limited by available resources. Access to the conservation areas is controlled in visitor areas by park rangers; however, backcountry access by sportsmen is generally unmonitored. National Park officers sporadically inspect sportsmen at recreation access gates but have inadequate personnel to provide full time monitoring for a park with 137 mi (220 km) of coastline; 484,200 acres (196,000 hectares) in Florida Bay and the Gulf of Mexico; 572,200 acres (231,500 hectares) of sawgrass/freshwa-

FIGURE 6. Only seed capsule in the extant *Trichocentrum undulatum* population in 2006. Photo by Wesley Higgins.

FIGURE 7. *Trichocentrum undulatum* recruits (seedlings and young plants) in the healthy areas of buttonwood forest. Photo by Wesley Higgins.

FIGURE 8. This specimen of *Melanagromyza miamensis* is deposited in the Florida State Collection of Arthropods. Photo by Gary Steck.

ter marsh; 230,100 acres (93,100 hectares) of mangrove forest; and 220,000 acres (89,000 hectares) of coastal areas (Cape Sable, river headwaters).

HABITAT RESTORATION. Although the state of Florida (USA) has adopted the IUCN guidelines for applying conservation criteria for animals, plants are regulated by the Florida Department of Agriculture and Consumer Services (FDACS) as endangered, threatened, or commercially exploited. For instance, although the buttonwood forests in Everglades National Park are in decline, the species, *Conocarpus erectus,* is not listed by FDACS; but is listed as Secure in South Florida by IRC since it occurs in most coastal counties of peninsular Florida. The orchid, *Trichocentrum undulatum,* is listed as Critically Imperiled by IRC and Endangered by the State of Florida; and the fly *Melanagromyza miamensis* is considered Data Deficient by authors. Presumably, if hydrological restoration is successful, ground water levels will be raised, wet season flows returned to transverse the everglades and fire intensities decreased, all to a degree that improves growing conditions for native species. The interaction between species is critical in habitat restoration. The fly reproduces in the orchid, which grows in the forest. Thus the Federal and State governments have made hydrological restoration a priority.

FUTURE INTERVENTION. The Comprehensive Everglades Restoration Plan calls for restoration of hydroperiods as the initial step in habitat restoration. The inter-dependency of species, limits which interventions are appropriate (tree – orchid – fly). For example, application of a systemic insecticide may be beneficial to the orchid reproduction but detrimental to the fly. Researchers are proposing both in-situ and ex-situ intervention: hand pollination and reintroduction. Researchers can hand pollinate orchid flowers to increase fruit set, which may increase the availability of seed, depending on the degree of reproduction interruption by the fly. Ex-situ propagation of orchid seed to produce propagules for population augmentation may increase population if forest decline can be reversed. MSBG and IRC are investigating symbiotic germination of orchid seed using myrrchoizza fungi. Having the symbioant present may increase reintroduction success. Although the fly is data deficient, increasing the orchid population may also assist fly reproduction. This example of interaction between two species, one with widespread distribution but Critically Imperiled locally (*T. undulatum),* and a possibly endemic species (*M. miamensis*), is used to demonstrate the complexity of developing a management plan.

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LITERATURE CITED

Gann, G. D., K. A. Bradley & S. W. Woodmansee. 2002. Rare plants of South Florida: Their history, conservation, and restoration. Institute for Regional Conservation. Miami, Florida.

Comprehensive Everglades Restoration Plan (CERP). http://www.evergladesplan.org/. Accessed: May 2006.

Seavey, R. & J. Seavey. 2006. Mule ear orchid new fly association**.** http://www.seaveyfieldguides.com/. Accessed: May 2006.

Spencer, K. A. 1973. Agromyzidae of Florida 7:43, in Arthropods of Florida. Florida Department of Agriculture and Consumer Services, Gainesville, Florida.

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IN VITRO PROPAGATION OF *CATTLEYA* LINDL. AND *LAELIA* LINDL. SPECIES

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KEY WORDS: micropropagation, *Laelia*, *Cattleya*, protocorm, *in vitro*

Nowadays many wild species of South American's orchids are under threat of extinction from over-collection and habitat destruction. Many tropical native orchid species were propagated in the National Botanical Garden of National Academy of Sciences of Ukraine through a range of asymbiotic seed germination techniques and tissue culture procedures aimed to preserve a number of individuals under artificial conditions in glasshouses in the temperate zone, with the aim to protect these species from complete extinction. Our orchid collection includes plants of *Cattleya* and *Laelia* species. Some of these species are rare in the wild. To protect them from extinction the methods of propagation should be developed.

Thus the objective of this study was to elaborate a methods for mass rapid seed and clonal propagation *in vitro* of five *Cattleya* species (*C. aclandiae* Lindl., *C. bowringiana* Veitch., *C. granulosa* Lindl., *C. intermedia* Graham. ex Hooker., *C. percivaliana* O'Brien.), and seven *Laelia* species (*L. anceps* Lindl., *L. lobata* (Lindl.) Veitch., *L. lundii* Rchb. f. et Warm., *L. mantiqueirae* Pabst., *L. purpurata* Lindl., L. *rubescens* Lindl., *L. sincorana* Schltr.), to study the development of protocorms and seedlings in vitro[a, b].

To obtain seeds, flowers of the studied species were self-pollinated by hand under glasshouse conditions in the National Botanical Garden. The seeds of *L. rubescens* were received from the Main Botanical Garden (Moscow, Russia) in January 2001.Our preliminary results showed that the capsules of orchid studied usually, ripened about 9-10 months after pollination, but seeds from unripe capsules can germinate *in vitro* much more earlier. Therefore the seeds from immature capsules about halfway of maturation were used for sowing on Knudson medium modified by addition of 2 mg/l peptone, 50 mg/l potassium hummate, 1 mg/l activated charcoal.

Seeds from dehisced capsules were sterilized in 10% Clorox for 15 to 20 min, in 15% H_2O_2 for 10 min, and then rinsed two times with sterile distilled water. Undehisced immature capsules were surface-sterilized as follows: rinsed with tap water for five minutes, then flamed after spraying with 96% ethanol. Capsules were cut open and seeds were transferred to cultivation media.

The cultures were incubated in 250-ml Erlenmeyer glass flasks in the laboratory at 25-26^{0}C, photoperiod 16h and relative moisture of air 70%. After sowing of seeds, flasks were inspected for seed germination and pathogen infection every seven days.

Seed germination of Cattleya and Laelia species on average began after 2 or 4 weeks of culture (tab. 1). Developing embryo exceeded initial size of embryo in 2 or 4 times, forming protocorms which shape is species-specific.

The protocorms were formed by undifferentiated highly vacuolated parenchyma cells, which are surrounded by a single layer of epidermal cells. For proliferation of protocorms the MS medium supplemented by the addition of 5 mg/1 BAP and 2 mg/1 NAA was used. Process of protocorm formation with many meristematic apices was highly influenced by the

[a] NBG's collection of tropical orchids was registered in Administrative organ CITES in Ukraine (notification ? 6939/19/1-10 from 23.06.2004).

[b] The name of species are given according to C. Withner 1990, 1991.

TABLE 1. The terms of seeds germination and seedlings propagation of *Cattleya* and *Laelia* species.

Nº	Species	Start germination	Seedlings formation	Ex vitro transplantation
			days	
1	*Cattleya acladiae* Lindl.	86	260	350
2	*Cattleya bowringiana* Veitch	16	134	300
3	*Cattleya granulosa* Lindl.	10	120	400
4	*Cattleya intermedia* Graham ex Hook	97	323	871
5	*Cattleya percivaliana* O'Brien	40	260	539
6	*Laelia anceps* Lindl.	95	287	1458
7	*Laelia lobata* (Lindl.) Veitch	11	240	1080
8	*Laelia lundii* Rchb.f. & Warm.	60	135	300
9	*Laelia mantiqueirae* Pabst	5-7	90	915
10	*Laelia purpurata* Lind.	27	191	394
11	*Laelia rubescens* Lindl.	17	270	930
12	*Laelia sincorana* Schltr.	80-90	270	400

level and distribution of exogenic hormones in cultural media. Later, in apical zone of protocorms the formation of apex and leaf primordia of shoot was observed. This was accompanied by the differentiation of procambial and conducting bundles.

Seedling formation, in average, took about 700 days (tab. 1). By the time seedling were transferred to glasshouse culture conditions. More over, seedling (and plantlets) also may be use as a secondary explants to enlarge the coefficient of propagation.

It should be noted that the process of ontogenesis of *Laelia* and *Cattleya* seedlings are quite similar. The differences are only in terms and details of development. The development of individuals in juvenile population of seedlings is not similar in vitro. The investigation has shown, that the seedlings have some pathways of ontogenesis in vitro. It was established, that at the initial stages of seedlings ontogenesis in vitro go through two basic patterns of development. For majority of species studied formation of secondary protocorms on primary protocorms are typical. The number of the seedlings, which have been developing through each of patterns, depends not only on abiotic factors complex. It is defined by interaction: a genotype ↔ a nutrient medium composition.

Different methods of clonal plant micropropagation of *Cattleya* and *Laelia* cultivars genotypes were developed in vitro culture. For propagation young growing shoots of 10-15 cm in height were selected. Apical and lateral shoots were used as initial explants. The buds of basal part of shoot had the highest morphogenetic potencies. Buds of some species after 6 months of cultivation on nutrient medium formed about 70 protocorms, while the middle part of shoot formed not more than 10-15 protocorms. So, we determined that apical meristems of young shoots of all genotypes studied are inert under cultivation in vitro. It doesn't develop later. Explants ability to regenerate depends on phase of plant-donor development. Buds found in May-June form protocorms more intensively and quickly, that is provoked by increasing of phytohormonal complex activity of plants in this period. We used different modifications of nutrient media for cultivation of Cattleya's and Laelia`s explants. Optimal medium for protocorm proliferation was MS with 5 mg/1 BAP, 2 mg/1 NAA, 100 mg/1 peptone, 15% coconut milk, 1.5 g/1 activated charcoal. More intensively protocorms were formed in darkness. The most active zones of protocorm formation are bases of leaf primordiums and bud squamules. As a rule 4-5 meristematic centers with lots of protocorms form simultaneously, they can be divided and cultivated.

Our research was carried out to examine the suitability of the basal and lateral buds of young shoots as explants for mass rapid clonal propagation of

species studied. The size of these explants did not exceed 0,5-1,0 cm. It was established that basal buds of shoot have the highest morphogenetic potencies.

Thus, combining some methods of seed and micropropagation in vitro we can get planting material of these beautiful ornamental plants. Effective method of Cattleya and Laelia plants micropropagation is induction of protocorm formations on leaves of plantlets and seedlings. Leaves were carefully separated from stem and cultivated on MS with 2 mg/1 BAP, 0.3 mg/1 NAA, 15% coconut milk. After one month at the base of leaves, at first from epidermal tissues form numerous of protocorms, followed by shoots formation.

Alla Lavrentyeva was educated at the National Agrarian University in Kiev, Ukraine. Since 1975 she works in M.M. Grishko National Botanical Garden of NASU as a senior researcher at the seed and micropropagation lab, where she received her PhD degree in biology on Optimization of microclonal propagation of Cymbidium hybr. in vitro. She published more than 100 articles and some books on this topics.

Roman Ivannikov was born in 1974 in Romni, Ukraine. Then he was educated at the Tarasa Shewscenka National University of Kiev, Ukraine. Now he works at M.M. Grishko National Botanical Garden of NASU as a senior researcher at the seed and micropropagation lab, where he received his PhD degree in biology on Biology of development of the species of genus Laelia Lindl. (Orchidaceae Juss.) under the conditions of greenhouse and in vitro. He interesting in Reproductive Biology and Conservation of some tropical genus of Orchidaceae in vitro. He published more than 30 articles on these topics.

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THE ROLE OF CITES RESCUE CENTERS IN ORCHID CONSERVATION: CONCERNS AND QUESTIONS RAISED BY THE COLLABORATION ON AN ENDANGERED SLIPPER ORCHID (*PAPHIOPEDILUM VIETNAMENSE* O. GRUSS & PERNER)

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KEY WORDS: CITES, *Paphiopedilum vietnamense*, Vietnam

The Convention on the International Trade in Endangered Species (CITES) is an international treaty currently adopted by 169 member countries to regulate international trade in over 30,000 species of animal and plants. Plants that are not transported in accordance with CITES requirements may be either denied entry, and sometimes abandoned, or subject to seizure by enforcement officials in importing countries. Instead of being destroyed, abandoned or confiscated plants may be returned to the range countries or sent to CITES Rescue Centers, which are public museums or botanical gardens in member countries where the plants are cared for and cultivated. The role of these rescue centers has traditionally been educational, with the CITES plants used and/or displayed to the public in an exhibition or show aimed at raising public consciousness about orchids and their conservation issues. In many cases, the plants placed in rescue centers are unusual, extremely rare or even new species, and may consist of multiple wild-collected plants exhibiting natural population variability. These ex-situ 'populations' are often sufficiently diverse to maintain a vibrant and vigorous gene pool within a captive breeding scenario. Increasingly, as habitats are disturbed or destroyed, it has become apparent to many well meaning staff of botanic gardens and arboreta, particularly CITES rescue centers, to do more than provide care and display these species. Rescue centers should give consideration to the value of the genetic material entrusted to them, which over time and with the participation of multiple centers will become progressively more and more important for establishing co-operative *ex situ* breeding groups and might eventually serve as a source of plants to repopulate degraded habitats and reduce collection pressure. Propagation of these plants often involves resources, such as laboratories, related equipment and expertise unavailable at public gardens and could provide an opportunity for collaboration with commercial growers and private citizens that can easily provide these facilities and expertise. Particularly for showy, commercially desirable plants, such collaborators are often anxious to offer their services.

An example of this type of collaboration was the legal propagation and distribution of *Paphiopedilum vietnamense* from nine plants that arrived at the U.S. Botanic Garden in 1999 (the same year the species description was published). These plants were part of a larger seizure at the port of Seattle by the U.S. Fish and Wildlife Service. The U.S. Fish and Wildlife Service initially contacted the government of Vietnam which declined to repatriate the orchids. As a result, the orchids remained at the U.S. Botanic Garden under the sole jurisdiction of the U.S. Government. Subsequently, The Fish and Wildlife Service permitted a private orchid growing facility access to the *P. vietnamense* housed at U. S. Botanic Garden. Sibling crosses were made from the surprisingly genetically varied collection. The seeds were subsequently grown *in vitro* at the New York laboratory and flasks offered for sale with the condition that plants be offered to other botanical institutions. The benign intent of

this collaboration was to offer legal plants in quantities that would serve to reduce collecting pressure on wild populations. Unfortunately, despite successful cultivation of the seedlings and the best of intentions, these propagated plants have still not been distributed to their intended recipients. This is attributable more to the vicissitudes of life than to anyone's greed or bad intent, but is indicative of the need for accountability when such collaborations are undertaken. Beyond this however, certain legal and ethical concerns are generated when such partnerships are entered into. Activities involving CITES Appendix-I plants need to be approached with the utmost consideration of the implications for the species' conservation.

There are three major conservation concerns that must be considered when making progeny of seized plants available commercially. First, the potential exists for unscrupulous collectors to continue to import illegally in order to obtain plants once they are introduced into commerce. The plants may actually be smuggled purposely to get legal plants in the trade. If not closely regulated, the legal plants are merely a smokescreen for continued pillaging of wild populations that threaten the species ultimate survival in its natural habitat. Second, regardless of whether the country of origin declines the return of the plants, they may still be opposed to the commercialization of their native species for profit, particularly profit that yields no benefit to the native country. Third, while the propagation and release of plants to commerce may reduce collection pressure, it does not eliminate all illegal trade if demand is high. Thus, the burden on law enforcement increases or becomes impossible as it becomes more difficult to separate legally and illegally received plants. If no legal plants are available, identification of the illegal is greatly simplified and law enforcement can be administered in an unclouded atmosphere.

This leads us to the question of what CITES rescue centers should do when they receive such desirable plants. The successes and concerns raised by this collaborative effort can serve as a guide for creating a protocol for conservation and propagation programs for any endangered orchid species that might be received by rescue centers in the future. In the meantime, the most significant role for Plant Rescue Centers continues to be the use of plants for display aimed at public education about conservation issues as well as the provision of the best possible horticultural maintenance and in-house propagation of the plants under their care, especially those taxa that are rare and endangered. Ideally, future conservation projects will involve in situ cooperation with the range country. Such efforts support the continued survival of these species in the habitats where they evolved and belong. And the efforts of CITES Rescue centers, instead of inadvertently contributing to wild extirpation can be more constructive, potentially reintroducing artificially propagated but genetically viable plants in protected areas when appropriate.

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Robert R. Gabel has worked in the U.S. CITES Scientific Authority since1991, and has been Chief of the office since 2001. He has been employed by the U.S. Fish and Wildlife Service for 27 years, primarily working on endangered species research and captive propagation, and international wildlife trade (including plants). He is a member of the IUCN Orchid Specialist Group, the Conservation Committee of the American Orchid Society, and currently is the North American Regional Representative on the CITES Plants Committee.

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MOLECULAR IDENTIFICATION AND GENETIC STUDIES IN PERUVIAN PHRAGMIPEDIUMS

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KEY WORDS: *Phragmipedium,* DNA markers, ITS sequences, AFLP sequences, *Phragmipedium kovachii, Phragmipedium boisserianum*

Peru has inherited one of the greatest biodiversities of the planet. The orchid genus *Phragmipedium* has several representatives in the country. They are listed in Appendix 1 of CITES, restricted from international trade.

Molecular analyses can be used as database for legal and forensic determinations. Phylogenetic analyses distinguish species in the genus *Phragmipedium* with low sequence divergence within sections. DNA markers of *Phragmipedium besseae, P. besseae var flavum, P. boisserianum, P. caricinum, P. caudatum, P. kovachii, P. longifolium, P. pearcei, P. schlimii and P. wallisii* were studied.

As expected, the individual and combined analyses demonstrate the distinctiveness of the molecular sequence data of *Phragmipedium kovachii*. An elucidation of the systematic in sections *Micropetalum* and *Schluckebieria* is presented. Our results also propose a close phylogenetic relationship of *Phragmipedium boisserianum* to section *Himantopetalum*.

Dendograms with AFLP (nuclear ADN) technique and ITS (internal transcribed spacer of nuclear ribosome) techniques can contribute to establish the taxonomy of *Phragmipedium kovachii* related to other species.

A well vouchered database of species and hybrids of Phragmipediums is under construction to determine the illegal origin of plant material by using DNA sequence data.

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WORKING TOGETHER FOR ORCHID CONSERVATION – – THE NATIONAL BOTANIC GARDENS, GLASNEVIN AND BELIZE BOTANIC GARDENS

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KEY WORDS: Belize, collaboration, capacity building

Introduction

The National Botanic Gardens, Glasnevin (NBGG) and the Belize Botanic Gardens (BBG) have been involved in Belizean orchid research since 1997. Staff from NBGG had travelled to Belize on two prior occasions with the purpose of collecting living specimens of orchids, bromeliads and cacti, along with seed of other plants to add to the existing glasshouse collections at the Gardens. During the expedition of 1996 the Glasnevin team met Ken duPlooy who had gathered a substantial orchid collection for what was to become Belize Botanic Gardens the following year. The 10 years of collaboration between the two gardens has substantially increased the knowledge of Belize's orchid flora and improved the capacity of BBG to identify and cultivate the country's native orchids.

Knowledge of the orchid flora

The knowledge of the orchid flora of Belize has been amassed from many expeditions by various institutions, both native and foreign, over the last century. The most comprehensive documents on the subject include *Orchids of Guatemala* (Ames & Correll 1952), *Supplement to Orchids of Guatemala and British Honduras* (Correll 1963), *A new species and new records of Orchidaceae for Belize* (Adams & Cribb 1985), *An annotated list of orchids of Belize* (Catling & Catling 1988) and *Native orchids of Belize* (Adams et al. 1995). The latest publication to list the orchids that occur in Belize is a *Checklist of the vascular plants of Belize* (Balick *et al.* 2000). This and the *Native orchids of Belize* differ little in the species listed other than the former publication includes *Cattleya skinneri* Bateman and *Oeceoclades maculata* (Lindl.) Lindl., excludes *Pleurothallis barbulata* Lindl. and some nomenclature changes. Otherwise by 2000 the list of species included for Belize totalled 279 species. For the purpose of this paper and various statistics within, the authors accept that 279 is the figure of the orchid flora in 2000. Further nomenclature will follow the World Checklist of Monocotyledons and any exceptions will be noted.

Additional knowledge of the orchid flora

In 2001 the results of the almost yearly joint expeditions had accumulated and were published in *Additions to the orchid flora of Belize, Central America* (Sayers & duPlooy 2001) bringing the total orchid species recorded to 298. The additions included a recently described *Pleurothallis*, *Pleurothallis duplooyi* Luer & Sayers from a collection in the Toledo District (Luer 2001).

The joint garden expeditions have concentrated on this area as over 65% of Belize's orchid species can be found in this southernmost district. Not surprisingly, the majority of the new records have occurred in the Toledo district, especially around the not easily accessed Little Quartz Ridge. Toledo's orchid rich forests are home to most of the recently published records like *Cochleanthes flabelliformis* (Sw.) R.E. Schult., *Cranichis muscosa* Sw., *Lepanthopsis floripecten* (Rchb.f.) Ames, *Maxillaria cobanensis* Schltr., *Macroclinium paniculatum* (Ames & C. Schweinf.) Dodson, *Platystele ovatilabia* (Ames & C.

Schweinf.) Garay, *Pleurothallis deregularis* (Barb. Rodr.) Luer and *Specklinia spectrilinugis* (Rchb.f.) Pridgeon & M.W. Chase (Sayers & duPlooy 2003). Recently we have verified *Platystele pedicellaris* (Schltr.) Garay and *Scaphosepalum microdactylum* Rolfe, both collected in the Columbia River Forest Reserve of Toledo.

Platystele pedicellaris (Schltr.) Garay, Orquideologia 9 (2):120. 1974. *Pleurothallis pedicellaris* Schltr.

Columbia River Forest Reserve, Toledo District; epiphytic in wet broadleaf forest, 24/1/2004, *B. Sayers 04/1241.*

Scaphosepalum microdactylum Rolfe, Bull. Misc. Inform. Kew. 1893: 335.

Pueblo Viejo, Toledo District, epiphytic in wet riverine forest, 16/4/1998, *B. Sayers 98/590.*

No less fascinating are the high altitude, quasi-mist forests of Mount Margaret in the Cayo District, which, over the years has been the location for many of the new records such as *Acianthera johnsonii* (Ames) Pridgeon & M.W. Chase, *Dresslerella powellii* (Ames) Luer and *Kegeliella atropilosa* L.O. Williams and A.H. Heller (Sayers & duPlooy 2003). On the same small mountaintop we have recently collected *Dichaea trulla* Rchb.f. and *Stelis convallaria* (Schltr.) Pridgeon & M.W. Chase.

Dichaea trulla Rchb.f., Beitr. Orch. Centr.-Am. 104. 1866. *Epithecia trulla* Schltr., *Dichaeopsis trulla* Schltr.

Mount Margaret, Cayo District, epiphytic in quasi-mist forest, 2/2/2004, *B. Sayers 04/1250.*

Stelis convallaria (Schltr.) Pridgeon & M.W. Chase Lindleyana 16(4): 262 2001. *Pleurothallis convallaria* Schltr.

Mount Margaret, Cayo District; epiphytic in quasi-mist forest, 1/2/2004, *B. Sayers 04/1244.*

Herbarium specimens of orchids collected in Belize are an additional source of information. Indeed when we first encountered the leafless *Campylocentrum poeppigii* (Rchb.f.) Rolfe we had no idea that a specimen collected by William A. Schipp (Schipp 339) housed in Missouri existed. This specimen had escaped the attention of documenters of the orchid flora for many decades. Robert Dressler's work on *Sobralia* has revealed a specimen of the *S. amparoana/bradeorum/warscewiczii* complex collected in the Stann Creek District.

Two other taxa worth a mention are a species of *Pelexia* and *Scaphyglottis*. The *Pelexia* was first encountered in 1997, on the first visit to the Little Quartz Ridge area of the Southern Toledo District. The rosette of deep purple leaves was collected but it failed to adapt easily to cultivation and did not thrive. It was not until 2004 that the plant was successfully flowered and photographs and a specimen taken. Two names have been suggested, *Pelexia callifera* (C. Schweinf.) Garay and *Pelexia gutturosa* (Rchb.f.) Garay. The former has a distribution of northern South America whilst the latter is Central American. The specimen has yet to be deposited in a suitable herbarium but we expect that the latter is the correct name for the taxon. The other confusing species is the *Scaphyglottis*. Tentative efforts at identification have placed it close to *Scaphyglottis tenella* L.O. Williams, a species recorded from Guatemala, Costa Rica, Nicaragua and Panama. Again the specimen has yet to be examined by someone with a familiarity for the genus, and it could possibly be undescribed (R. Dressler, pers comm.).

Distribution and conservation information

Even though the distribution of *Pelexia* is taken into account for the tentative determination of the unnamed specimen, Belize has a element of it's flora that does not occurr in neighbouring countries. *Native orchids of Belize* points to two species, *Maxillaria discolor* (Lodd. ex Lindl.) Rchb.f. and *Koellensteinia tricolor* (Lindl.) Rchb.f. The former is found outside Belize in Nicaragua, French Guyana, Guyana, Suriname, Venezuela, Bolivia, Peru and Brazil whilst the latter in Guyana, Peru and Brazil. Certain of the new records show this disjunctive distribution. Outside of Belize, *Dresslerella powellii* (Ames) Luer is only known to occur in Panama and *Phloeophila peperomioides* (Ames) Pridgeon & M.W. Chase only in Costa Rica. It may be the case that further investigations in neighbouring countries will reveal the presence of these species.

Similarly, exploration in Belize has proven wider

distributions of some orchids currently known to occur in only certain districts. Upon each successive expedition to the northern districts of Corozal and Orange Walk, orchids previously not recorded for these districts are found indicating a need for further research. The NBGG and BBG have expanded the knowledge of the Belizean orchid flora, not alone in numbers but in quantity and location also.

To be effective, conservation efforts need to have an information base – we need to know what needs to be conserved before we can make suitable efforts to do so. The two gardens are dedicated to the conservation of Belize's orchid flora not only in filling this need but plans are also being put in place to propagate sensitive species from seed.

At first glance it would seem that Belize's orchids would scarcely need protection. A huge portion of the country, 44% (1.2 million hectares) of land and sea are under protective status. Belize is also party to International conservation conventions such as the United Nations Convention on Biological Diversity and the Convention for International Trade in Endangered Species of Fauna and Flora. However there are significant threats ranging from individuals involved in exporting wild collected orchids to private collectors, to lack of enforcement of laws protecting orchids, to the ever growing threat of climate change.

Even the protected status of the country is also a dubious comfort as recently 500 acres of Cayo's San Antonio National Park was de-reserved, in Sstann Creek, Mayflower Bocawina National Park was de-reserved by 400 acres and in Toledo an International Oil Company has been given permission to begin seismic testing and subsequent oil drilling in the Sarstoon/Temash National Park.

The two Gardens, in their commitment to orchid conservation will continue their field research in Belize. To assist in promoting the orchid flora among native Belizeans and the substantial amount of visitors to the country, they are producing a small format guide to some of the more notable species of orchids. Recent developments at NBGG has seen the establishment of an orchid propagation laboratory where seedlings will be germinated to bulk up ex situ collections and act as a source of material for the future.

LITERATURE CITED:

Ames, O. & D.S. Correll. 1952. *Orchids of Guatemala and Belize*. Dover Publications, New York.

Adams, B.R. & P.J. Cribb. 1985. A new species and new records of Orchidaceae for Belize. Kew Bull. 40 (3): 635-642.

Catling, P.M. & V.R. Catling. 1988. An annotated list of orchids of Belize. Orquídea (Méx) 11: 85-102.

Luer, C.A. 2001. Miscellaneous new species in the Pleurothallidinae Rev. Soc. Bol. Bot. 3 (1/2): 48 Santa Cruz

Sayers, B. & H. duPlooy. 2003. Additions to the orchid flora of Belize, Central America. Lankesteriana 8: 1-3

Balick, M.J., M.H. Nee & D.E. Atha. 2000. Checklist of the vascular plants of Belize with common names and uses. Mem. New York Bot. Gard. 85.

McLeish, I., N.R. Pearce & B.R. Adams. 1995. *Native orchids of Belize.* AA Balkema Publ., Rotterdam and Brookfield.

World Checklist of Monocotyledons (2006): The Board of Trustees of the Royal Botanic Gardens, Kew. Published on the Internet, http://www.kew.org/wcsp/monocots, accessed October 30, 2006.

Brendan Sayers oversees the glasshouse collections at the National Botanic Gardens, Glasnevin, Dublin, Ireland. He is particularly interested in the study of orchids, especially those of Belize and Ireland.

Heather duPlooy is the Curator of Belize Botanic Gardens. Heather took over management of the gardens, from her father, garden founder, Ken duPlooy in 2000. Her major achievements since that time have been the development of public and youth education programs in sustainable and organic horticulture and conservation awareness run by the gardens. She is actively involved in developing the organic industry of Belize through her work with the Belize Organic Producers Association and is dedicated to continuing her father's vision of making the Belize Botanic Gardens a useful resource for the country.

Brett Adams has been working at Belize Botanic Gardens since 2002. He is currently one of two Foremen that oversee horticulture and collections at the Garden. He also developed and manages the Garden's information system for plant records.

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THE IMPORTANT ROLE THAT THE BOTANICAL GARDEN OF THE NATIONAL AUTONOMOUS UNIVERSITY OF MEXICO PLAYS IN THE CONSERVATION OF MEXICAN ORCHIDS

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RESUMEN. El Jardín Botánico del Instituto de Biología de la Universidad Nacional Autónoma de México ubicado en la Ciudad de México, México; asentado en terrenos de origen volcánico en un matorral xerófito; tiene el propósito de mantener colecciones de plantas vivas representativas de la diversidad vegetal de México, que sirven de apoyo a la investigación, conservación, educación y Divulgación de la diversidad botánica. El Jardín posee una colección Viva de Orquídeas, tanto epífitas como terrestres, en la que se han llevado a cabo diferentes actividades. Con este trabajo se enfatiza el papel que juega el Jardín Botánico de la UNAM, en la conservación de la familia Orchidaceae de México.

KEY WORDS: Mexico, Botanic Garden, Orchidaceae, conservation, endemic, *ex situ*

Mexico is located in the southernmost part of North America (14° 32' 27"; 32° 43' 06" north latitude and 86° 42' 36"; 118° 22' 00" west latitude).The country has an area of 1,964,375 km^2 (INEGI 2006) and is divided by the Tropic of Cancer into two halves. The northern region is mostly arid, temperate, with its climate strongly influenced by North American continentality. In contrast, southern Mexico is humid and tropical due to its lower latitude and the maritime influence, and has a more rugged topography (Challenger 1998). Mexico is situated where two biogeographic regions meet, neartic and neotropical. It has complex topography as a result of its intricate geological history. These two facts are the cause of such enormous diversity in Mexico (Neyra y Durand 1998). Biological diversity in Mexico is one of the highest in the world (4º mega diverse country) and contributes, with other 12 countries to around 60 to 70%, of global diversity (Alvarado 2000) (Fig. 1).

The conservation of the biodiversity is an integral system of activities to assure the existence and natural reproduction of the populations in their natural habitat (*in situ*) and out of their habitat (*ex situ*) as Botanical Gardens do.

The Botanical Garden of the Biological Institute of National Autonomous University of Mexico (UNAM) is situated geographically between parallel 19° 20' 23" and 19° 13' 45" north latitude and the meridians 99° 08' 26" and 99 °14' 37" east long. It is to the South of Mexico City in the area known as The Pedregal of San Angel, a volcanic site formed 2500 years ago by the Xitle volcano complex (Fig. 2).

This Botanical Garden has the intention to maintain collections of representative living plants of the vegetal diversity of Mexico. These collections, serve as support for research, conservation, education and spreading of the botanical diversity. The Garden has

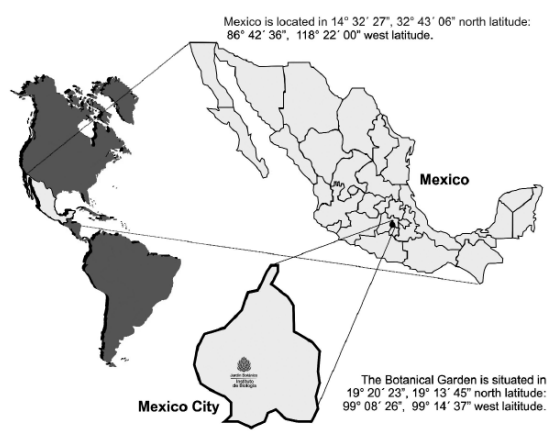

FIGURE 1. Location of Mexico in the world. Ilustration J. Saldivar.

FIGURE 2. A look of the Botanical Garden, UNAM. Ilustration J. Saldivar.

plants of arid, tempered and warm-humid zones. From these last two zones, there is a living orchid collection, including epiphytes and terrestrial species (Fig. 3). This collection was started in1960, and since then, different activities related to the conservation of biodiversity have been carried out, such as:

1. – There are 1,200 species of Mexican orchids (Hagsater *et al*. 2005).One of the most outstanding features of the Mexican orchid flora is the high proportion of endemic species. There are 444 endemic species o subspecies corresponding to about 40 % of the total recorded flora taxonomy of this country. This feature makes the Mexican orchid flora proportionally one of the richest in endemism among non tropical mainland countries, perhaps surpassed only by Brazil (Soto 1996).

The Botanic Garden shelter and protect under cultivation 71 genera and 176 species in 1216 specimens of Mexican species that have biological, ecological (*Epidendrum magnificun*), artisan (*Myrmecophila tibicinis*), flower industry (*Cattleya aurantiaca*), medicinal (*Cyrtopedium puntatum*), adhesive (*Govenia superba*), flavoring (*Vanilla planifolia*), comestible (*Epidendrum rigidum*), ornamentals (*Oncidium sphacelatum*), narcotic (*Prosthechea radiata)* importance. Each of the orchid pollination syndromes is represented with specimens: bee (*Lycaste aromatica*), fly (*Restrepiella ophiocephala*), moths (*Epidendrum parkinsonianum*), butterfly (*Epidendrum radicans*), and hummingbirds (*Elleanthus capitatus*). There are specimens belonging to the four subfamilies reported by Chase *et al* (2003): Vanilloideae (*Vanilla*), Cypripedioideae (*Paphiopedilum*), Orchidoideae (*Shiedeela*) and Epidendroideae (*Pleurothallis*).

FIGURE 3. Living orchid collection of the Botanical Garden, UNAM. Ilustration A. Téllez.

Mexican endemic species of orchids are safeguarded and kept, in addition to those species given the status of Probably extinct (E**),** In danger of extinction (P), Threatened (A), and Under special protection (PR) as stated in the Mexican Official regulations "NOM-059-ECOL-2001" SEMARNAT (2002). In this document 181 species are registered of which 72 are endemic and 109 not endemic. The Botanical Garden protects species of the category from A: [*Encyclia adenocaula* (endemic), *Bletia urbana* (endemic) and *Chysis bractensis*] and in the category of PR: [(*Vanilla planifolia* (endemic), *Prosthechea vitellina, Euchile citrina* (endemic) and *Laelia speciosa* (endemic)] (Fig. 4).

The objective of this *ex- situ* conservation is to assure the protection the species, while mantening the variability of these. The Garden has responsibility on these plants like deposit takers perhaps of the only germoplasma surplus of threatened species. For that reason, they are subject of special attention regarding their maintenance, horticultural and curatorial activities and propagation.

2. - Accomplishment of diverse studies of *in vitro* propagation, of some species with conservation problems, including the formation of somatic embryos from apex of protocorms (*Euchile maria, Trichocentrum carthagenenese);* multiple sprouting of knots, obtaining direct and indirect organogenesis from leaves *(Vanilla planifolia)*; regeneration of plants from callus, by seed (*Bletia urbana* y *Stanhopea tigrina*); by leave section (*Laelia speciosa*), symbiotic and asymbiotic germination (*Bletia campanulata, Dichromanthus aurantiacus* y *Dichromanthus cinnabarinus).*

With these propagation methodologies that do not damage wild populations, parts or complete seedlings are obtained that represent a heritage or bank of germoplasm *in vitro* and *ex vitro* as well as *ex situ*. The reintroduction of some terrestrial orchids to its habitat (*Bletia urbana* and *Dichromanthus aurantiacus*), is another activity directed to re-establish not only the vegetal structure, but also the operation of the ecosystems (Fig. 5).

3. - Observation and *in situ* monitoring of endemic species or species with conservation problems, in protected natural areas like "Ecological Reserve of the Pedregal de San Angel" located within the grounds of the UNAM, for studies of distribution, ecology and phenology of terrestrial orchids, registering one endemic species, *(Bletia urbana*) (Tellez 2002). The Botanical Garden has in shelter 86% of the registered terrestrial species for the Reserve (Fig. 6).

In the Reserve of the Biosphere, Barranca de Metztitlán, in Hidalgo, Mexico, species as *Laelia gouldiana*, endemic of the area and *Laelia speciosa*, endemic of Mexico, among others species, are being monitored (Fig. 7).

4. - During recent years the Mexican natural habitat has been transformed by heavy logging, agriculture, cattle raising, chemical pollution, wood fires and

FIGURE 4. *Laelia speciosa*, endemic species of Mexico and subject to special protection. Ilustration A. Téllez.

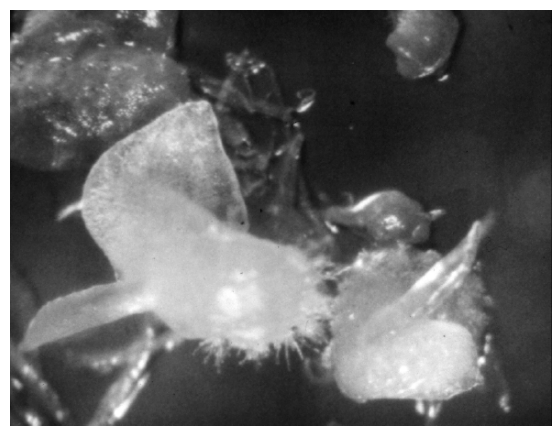

FIGURE 5. *In vitro* propagation of *Euchile citrina*. Ilustration A. Téllez.

FIGURE 6. *Bletia urbana* endemic species of Mexico and under special protection. Ilustration A. Téllez.

FIGURE 7. *Laelia gouldiana*, extinct and endemic species of Barranca de Metztitlan, Hidalgo.Mexico. Ilustration A. Téllez.

FIGURE 8. Rescue of orchids in Santa Marta, Veracruz, Mexico. Ilustration A. Téllez.

FIGURE 9. Workshop of orchid growing and propagation in Mexico City. Ilustration A. Téllez.

urban expansion – all these in addition to the extraction of species of commercial value. For this reason, the Botanical Garden have to rescue plants in zones that have been altered by urbanism, as is the case of Distrito Federal (inside UNAM campus), in which terrestrial orchids have been rescued (among which are *Sarcoglottis schaffneri, Dichromanthus aurantiacus, Habenaria novemfida*) and in Xalapa, Veracruz, Mexico, where Bletias are being rescued in places where highways are being expanded or rocks are being mined (Fig. 8).

5. - The collections of the botanical Garden and their biodiversity are means so that the people form an idea of the problematic and importance of conservation. An educative relation with its users must exist; because the collections by themselves do not work like tools of environmental education if they do not count on information that allows to interpret them; as labels, calendars, guided visits, preparation of materials like news bulletins, guides of routes, exhibitions, conferences, talks, courses and workshops.

Activities like workshops, courses and exhibitions that support the environmental education of people, from children to adults, having the objective to spread knowledge and to create environmental awareness. As an example of workshops there are those directed to cultivators, plant dealers, and public in general on the cultivation and propagation of orchids and one directed for children named "An orchid called Vanilla" (Fig. 9).

Finally, this work emphasises the importance and the role that the Botanic Gardens of the UNAM plays, in the conservation of the Orchidaceae family of Mexico.

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LITERATURE CITED

Alvarado, Z. A. 2000. Campamento biofilia. La biodiversidad. SOMEDICIT-SEMARNAP, México, D.F. 91 p.

Challenger, A. 1998. Utilización y conservación de los ecosistemas terrestres de México, Pasado, presente y futuro. CONABIO.México, D.F.847 p.

Chase M.W., K.M. Cameron, R.L. Barrett y J.V. Freudenstein. 2003. DNA data and Orchidaceae systematics: A new phylogenetic classification. Pp. 9-89 *in* K.W. Dixon, S.P. Kell, R.L. Barrett & P.J.Cribb (eds.). Orchid conservation. Natural History Publications (Borneo), Kota Kinabalu Sabah.

Hagsater, E., M. Soto Arenas, G.A. Salazar Chavéz, R. Jiménez Machorro, M. A. López Rosales & R.L. Dressler. 2005. Las orquídeas de México. Instituto Chinoín, México, 304 p.

INEGI(2006. http://mapserver.inegi.gob.mx/geografia/espanol/datosgeogra/extterri/frontera.cfm?c=154

Neyra G. & L. Durand S. 1998. Biodiversidad. Pp. 61-102 *in*: Conabio (comp.) La diversidad biológica de México. Estudio de país. CONABIO. México, D.F.

Rzedowski, J. 1978. Vegetacón de México. LIMUSA. México, D.F.432 p.

SEMARNAT. 2002. Norma Oficial Mexicana. Proy. NOM.059-ECOL-2001. Diario Oficial de la Federación, marzo. México, D.F.

Soto A.M. 1996. Mexico. Pp. 53-58 *in*: E. Hágsater& V. Dumont (eds.). Orchids. IUCN. Cambridge.

Tellez-Velasco, A. 2002. The Pedregal of San Angel and its orchids. Orchid Rev. 110 (1242): 25-29.

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ESTABLISHING AN ORCHID CONSERVATION ALLIANCE

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Introduction

Almost every orchid society and orchid grower realizes that orchids in the wild are in danger of disappearing. The simple growth of the human population dictates that spaces that once were subject only to the vagaries of nature are now impacted by the human need for space to live and grow food. As orchid lovers, what can we do ?

The San Diego County Orchid Society

About 15 years ago some of the members of the San Diego County Orchid Society (SDCOS) decided that we should support orchid conservation in some concrete way. At that time the Nature Conservancy as well as the Massachusetts Audubon Society in the United States were assisting with establishment of a conservation area in Belize known as the Rio Bravo Conservation and Management Area. The purchase of the land had incurred a significant debt by the Nature Conservancy and we decided to assist with retiring that debt and thereby ensuring the establishment of the conservation area. We decided to sell our excess plants to raise the money and asked the SDCOS to grant us a free sales booth at its spring orchid show and sale. To our pleased surprise we raised approximately US$4600 at our first sale in 1991. We repeated this activity for two more years, raising a total of about US$14,000 that was donated to the program for Belize. Subsequently we continued our fund raising efforts but we decided to support some other organizations. In 1996 we realized that our knowledge of projects worthy of support was limited and we decided to advertise for proposals. Since then we have supported between 3 and 8 projects per year. We have now expanded our sales to twice yearly and earn about $10,000 per year. Donations of quality plants for sale have been very generous. In most cases these

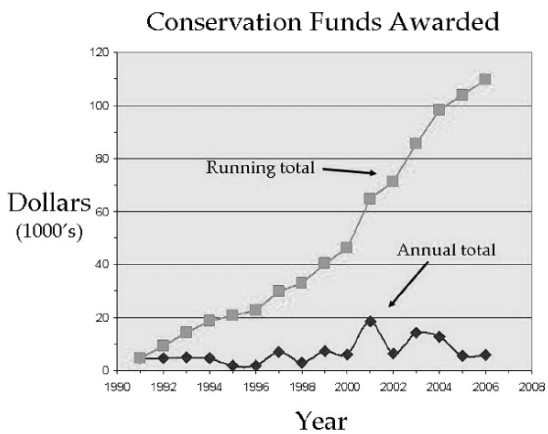

FIGURE 1. Annual and running total of funds donated for conservation projects by the SDCOS.

are simply extra plants grown by members of the SDCOS. We do receive some donations from local professional growers, from nurseries going out of business, and also occasionally from the estates of deceased members. Quite a few members of the society help with the work of repotting, caring for donated plants before the sales, and staffing the sales booth twice yearly. The annual and running total of funds donated for conservation projects by the SDCOS are shown in figure 1. A listing of projects supported can be found at the SDCOS web site www.sdorchids.com. The SDCOS continues to solicit applications for funding.

Potential applicants are encouraged to look at the list of projects already funded to see what sorts of projects the SDCOS does support and to contact Ron Kaufmann, Chairman of the SDCOS Conservation Committee at kaufmann@sandiego.com for more information.

The Orchid Conservation Alliance

Two of the authors (PST and RK) have been associated with the San Diego County Orchid

Society for many years. As we participated in the SDCOS conservation program we realized that if orchid growers in San Diego were so generous in their support of conservation that probably there were other growers outside San Diego who would be generous also. The question was how to reach them ? We considered reproducing the sales of donated orchids in other areas but the logistics of doing that from San Diego seemed insurmountable, given that we both had, and needed, jobs to support us and our orchid collections. Of course, we hope that orchid societies in other locales will be able to duplicate the success of the SDCOS program, but we could not do that ourselves. Thus we conceived the idea of establishing a new membership organization of orchid lovers devoted to orchid conservation. There are societies devoted to conservation of orangutans, whales, birds, plants, etcetera, and nature in general, but none that seemed to have a special focus on orchid conservation. Given that the circulation of the American Orchid Society's journal "Orchids" is above 22,000, it seemed that there was a significant pool of potential members. And so we decided on a name, the Orchid Conservation Alliance or OCA, gathered some directors and a scientific advisory board, defined an initial project, applied for incorporation in the state of California, and applied for tax exempt status from the US government. We decided that our primary purpose was the preservation of orchids in their native habitats. We realize that there are many aspects to orchid conservation, but it seemed to us that the root of the problem was the loss of habitat. Furthermore, preserving habitat preserves orchids not yet identified. Clearly, preserving habitat is not simple and defining which pieces of habitat to preserve with the means available can be difficult, but, frankly, there is no time to lose.

We have defined three criteria that must be met for a parcel of land to be targeted for preservation. These criteria refer to biodiversity, local involvement, and legal status. An appropriate orchid biodiversity must be present. We do not deny the conservation value of deserts and glaciers, but we are an orchid conservation organization. There must be demonstrable local involvement for protection of a parcel of land. Most of the orchid habitat we are likely to consider for protection is in the tropics and we are in North America. We are unlikely to be able to oversee the management of an area in a foreign country. Thus we seek local organizations to take ownership of any parcel of land we might support for preservation. And finally, there must be some legal mechanism for long term protection of the land. With these thoughts in mind the first project we undertook was to set aside land in Ecuador. Ecuador is well known for its astonishing orchid biodiversity. Ecuador has laid the legal basis for protecting against development because it has the means to establish conservation easements. And we were aware of the activities of Lou Jost in Baños. Lou and his associates have established the Ecominga Foundation for the express purpose of protecting local habitat. Thus we set as our first goal the raising of $10,000 to enable purchase of 100 hectares of land near Baños, Ecuador, by the Ecominga Foundation.

As noted above, we had decided that the Orchid Conservation Alliance should be a membership organization. We hope that we will be able to do a good enough job such that OCA members will be willing to donate money for more than one project. We started soliciting members by speaking at individual societies. Although this was successful in generating a small and enthusiastic initial membership, it was clear that we needed a more efficient means of contacting people. Enter Harold Koopowitz. As most people will know Harold Koopowitz is certainly committed to orchid conservation and he is also the Editor-in-Chief of the Orchid Digest. Through Harold and his associates at the Orchid Digest, especially Cynthia Hill and Steve Gollis on the publication committee, we were able to undertake a very successful membership campaign via the pages of the Orchid Digest. We now have over 120 individual members, more than 8 member societies, more than $13,000 in the bank, and successful completion of our first fund raising campaign. Most recently we earned an endorsement from the American Orchid Society.

As this article is being written we are considering where to focus our energies for a second project. There seem to be many opportunities. At present we are focusing on possible projects in Ecuador and Brasil. And we are planning our next fund raising campaign. There is certainly no lack of orchid biodiversity left to be protected. Individuals from any country interested in joining the OCA are urged to visit our web site at www.orchidconservationalliance.org or to contact Peter Tobias by email at peter@orchidconservationalliance.org.

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NOT A SINGLE ORCHID...

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We all know that the original intent of the Convention on International Trade in Endangered Species was for the protection of animals. Adding flora was an afterthought that seemed like a good idea at the time. But it has gotten out of hand, and CITES regulation is now deeply entrenched worldwide causing unnecessary expense, heartache and aggravation, not to mention contributing to corruption, smuggling and who knows what else. But I sincerely believe that CITES has not saved one plant from extinction since its inception.

Every thinking person believes in the concept of saving endangered species. We amateur and professional growers, taxonomists and educators, importers and exporters, individuals and organizations, all share a common goal, although the methods for achieving conservation have taken us on radically divergent paths. It is my opinion that CITES regulations, in practice, have proved to be no less than counterproductive to orchid conservation. Certainly, CITES is not the answer. Preventing the trade in animal life is one thing, but CITES was misguided in extending their policy to orchids with Appendix II. Wouldn't it be nice if the CITES authorities would seriously consider the difficulties that the treaty has caused the orchid world? I was once admonished by a famous orchid person to swallow my irritation and testiness, and softly approach the CITES situation with logic and reason, hat in hand as the only way to induce change. Really? I think the only way to get action is to raise holy hell over CITES injustice by confronting them forcefully. Maybe sit-ins won't work, but how about filing an international law suit in the World Court in The Hague?

A few years ago I began a petition that many people agreed with but would not sign. Why? Because CITES has injected fear in the orchid world. Fear of reprisal by CITES officers. Feeling their power, these officers in many instances behave badly by refusing to issue CITES certificates, or delaying them. Rather than functioning as public servants, some of the administrators behave as martinets. They are hired and paid by CITES in Switzerland and there's no way to change their status short of open revolt. Like Supreme Court judges in the United States, they are in for life. As we all know, Appendix I of CITES lists certain orchids that have been declared "endangered species" to prevent them from being transported across international borders. Each party to the treaty sets up its own system of enforcement. In the United States, the treaty is enforced by the U.S. Department of the Interior/Fish and Wildlife Service and the U.S. Department of Agriculture. As far as I know, none of the people in these organizations have bothered to study the orchids in the field to determine if they are, in fact, endangered.

For instance, every *Phragmipedium* species is included in Appendix I. Most of those who made, and those who enforce, the treaty have yet to visit the sites in situ. In Ecuador, one can see thousands of *Phragmipedium longifolium* plants growing in a single population. At a streamside location, large stands of many, many hundreds of plants of *Phragmipedium piercii* can be seen growing in clumps. *Phragmipedium besseae* populations range from Ecuador into Peru with plants numbering in the millions. Each of these orchid species grow at many locations. Most of the plants of *Phragmipedium kovachii*, labeled "the most important orchid find in the past 100 years" would still be in their natural habitat in Peru if plants had been exported *legally* to responsible growers for propagation. We know that many mature plants have been distributed worldwide… illegally. Without CITES. *Legal seedlings from flasks will become mature within a very short time.*

In Brazil, *Laelia jongheana* is also "protected" with Appendix I designation, yet they grow by the hun-

dreds of thousands. The only instance I know of where CITES has reversed their regulations is the removal of *Cattleya triane* from Appendix I. Many forms of this species have always been common in Colombia. It was great that CITES finally figured out it wasn't endangered.

Those familiar with the situation know that the international trade in orchids is a drop in the bucket compared to the loss of *millions upon millions* of orchids, along with their host trees and other plants, as the result of slash-and-burn agriculture. Apparently, this does not concern to CITES. And lip service does not save orchids that actually might be endangered. Based on the fact that there are substantial populations of all *Phragmipedium* species in Central and South America including *kovachii*, I believe that they should remove the species in this genus from Appendix I. Further, placement of *all other* orchids in Appendix II does not seem not realistic or constructive to me, and for those reasons and others, speaking for many people in the orchid world and myself, I feel it is time to kill, or at least modify, many of the restrictions for orchids.

Because orchids must have phytosanitary certification before being shipped or carried across some international borders, the quantity of orchids being shipped can still be checked and controlled at the time of these inspections. I remember the policy practiced in Jamaica prior to the advent of CITES that worked well. Collectors were limited to a maximum of five plants of any one species to be removed from the island. Orchids were checked and released upon completion of a plant health inspection and record of the species being taken.

I began illustrating plants and flowers in the *Oncidium* alliance in 1984. I was fortunate to receive plants from growers and dealers in Mexico and Central and South America and the Caribbean by mail, courier or in person. After completing nearly half the paintings for my book, *The Pictorial Encyclopedia of Oncidium*, along came CITES adding years to the work. That was the bad news. The book actually took thirteen years for me to complete the original edition. The good news is that to legally obtain plant material, I was forced to travel to many countries in Central and South America that I might never have visited.

I was once told by a CITES inspector when carrying a few sterile flasks to New York that charcoal should not be used in the gel medium because he could not see the roots. I could never understand why, in heaven's name, it was necessary for him to examine seedling roots in a sealed bottle? Ridiculous! And hybrids and seed are also now part of the inspection process in many countries.

Here's an idea: Importations, whether by individuals or commercial growers, can meet practical guidelines provided they pass sanitary inspection prior to shipping and an inspection at ports of entry. Yes, USDA inspectors *should* examine orchids and other plants for diseases, insects, and other pests. With such a simplified system of inspection, the nations of the world can expect the cooperation of importers, because no grower, private or commercial, wants to introduce possibly infected plants into their growing environment. Plant inspectors should be encouraged to work *with* responsible growers and scientists. How to change their attitude is a helpless cause.

A more flexible approach by those who have created as well as those who enforce CITES would allow desirable orchids to be imported for future propagation by responsible, certified growers. The subsequent availability of plants reproduced in numbers might then be sold at reasonable cost. Making them available might very well help protect orchids in their natural environment.

Here's where the idiocy of CITES shows its stupidity. A scientist wishing to send dried, pressed specimens of orchids or vouchers of flowers or other plant parts must go through the time and expense of obtaining CITES permits to carry or send material for their work. And a matching CITES at the receiving end must also be issued. To say the least, this is certainly not a productive application of CITES regulations. It hampers research and I think the complete removal of Appendix II restrictions would eliminate these problems. Recently, I prepared twenty small bottles with *Caucaea* vouchers in silica gel that I wanted to send to Mark Chase at Kew for DNA sequencing. I was refused a CITES in Quito. But I can take a bunch of cut flowers across international borders anywhere in the world. Frankly, I think this kind of organized inanity, coupled with abuse of power, needs to be exposed and discussed as often as possible to try to

wake up the CITES organization in hopes that someone there knows a little about orchids. Or is this a lost cause?

At the World Orchid Conference in Dijon a number of potential species exhibitors and vendors had their orchids blocked from entering France. No one is really sure what the reasons were—- or if they were legitimate reasons. But it seems mighty strange to me that only the people bringing orchid species were prevented from bringing in their plants by the French CITES and authorities. Brazil, Madagascar, Colombia, the Philippines, Peru and others spent much money for travel and shipping to no avail. The WOC management could not do anything to help.

CITES authorities should change their rulings on flora, orchids especially, to a more practical and sensible approach if they profess to save species (which they probably do not really care about). Orchids, trees, and other plants should be monitored; but it is my opinion that restrictive orchid rulings need to be re-examined and changed. Plants that are being destroyed by habitat destruction should be harvestable, and reasonable quantities of orchids should be allowed in trade. How about up to five of any one species per shipment per year? Removal of orchid species from Appendix II will advance orchid conservation and virtually eliminate the need for smuggling. But common sense and CITES do not seem to be compatible.

Harry Zelenko was born in 1928 in New York City. He was trained as an artist at the High School of Music & Art followed by the Art Student's League and courses in art at New York University. At the age of nineteen he became art director of the largest advertising agency in Connecticut. Zelenko Associates, a creative a design group, was founded in 1953. His work has received many professional awards and he has lectured and exhibited internationally. In New York, he built orchid greenhouses on the roof and terrace of his brownstone where he grew about three thousand orchids. In 1984 he began producing paintings for a book on orchids, and as Z A I Publications, published the first edition of *The Pictorial Encyclopedia of Oncidium* in 1997 with more than 850 paintings of plants and flowers. The second, revised edition followed seven years later. He moved to Ecuador and built two greenhouses to house a tripled collection of orchids. He is currently a member of the Greater New York Orchid Society and the Quito Orchid Society.

CONSERVATION AND INFORMATION TECHNOLOGIES

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WHAT IS BIBLIORCHIDEA?

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Introduction

Literature is still playing a major role in science and research, on one side because it is a documentation of research already done and a demonstration of the results. On the other hand it is also a record about recent research. At least for the time being, literature search is a part of any scientific work or project. Whoever has done literature search knows about the difficulties to reach an overview of the literature in connection with a project in an acceptable time and with acceptable effort. Computer technology today provides us with a large selection of very helpful tools to limit time and effort for literature search.

As explained above, literature still plays a very important role in science. In this context the term literature should not be defined too narrow, we have to accept a very broad collection of publications as literature. In botany – and in orchideology as a division of botany – we find:

- Scientific periodicals, occasionally with articles about orchids
- Scientific periodicals in the botanical field, occasionally with articles about orchids
- Orchid periodicals, with at least partially scientific content
- Society publications (Orchid Societies)
- Dissertations
- General floras or orchid floras
- Proceedings, abstracts and reports of congresses and symposiums
- Catalogues of all kind
- Travel and expedition reports
- Textbooks and basic research publications
- Bibliographies and Biographies
- Correspondence and letters

Botany as an independent scientific discipline is not very old. For centuries, botany was together with other natural sciences like zoology or geology always a part of medicine or part of a general study in natural sciences. The oldest European botanical literature was always very closely connected with medicine and pharmacology. In this time orchids were treated as a mere curiosity of nature and, in a limited number as part of pharmacological publications like herbals. Only a few publications devoted to orchids alone are known before 1800, after this time we see a fast increase in the number of publications devoted only to orchids. Today we have more publications about orchids than about any other plant family. Because of the more and more interdisciplinary connection of botany with other natural sciences like chemistry, biology or zoology the content of publications about or in connection with orchids has also become much broader. Like in many other sciences also in botany the pure generalist does not exist anymore. One result of the ongoing specialization in botany is the fact that the term botanist is no longer a synonym for classification, systematics or taxonomy. With this the existing literature becomes less and less manageable, and it is almost impossible to avoid repetitions in publication and research. For a good part, this is the result of the fact that today it is almost impossible to keep track of the enormous numbers of publications, in spite of the fact that we have tools like the internet at hand. One should think that based on the available data processing technology it should be possible to solve this problem in a simple way by building up databases or computerized bibliographies. This is not the case and the reason can be found in the philosophy and structure of such a database. We have to consider first the enormous variability concerning the content of orchid literature:

- Classification, systematics and taxonomy
- Nomenclature
- Genetics, molecular biology, DNA-analysis, enzymatics
- Anatomy and morphology
- Phytogeography, distribution, mapping

- Evolution, speciation, population dynamics
- Interactions plant - animal, pollination ecology
- Symbiosis, mycorrhiza
- Phytochemistry, fragrance, pigmentation, pharmacology
- Vegetation biology, habitat, ecology
- Physiology
- History
- Hybridization, breeding, commercial aspects
- Culture
- Propagation, micropropagation, tissue-culture
- Protection and conservation

Naturally this list is not complete by any means, but it shows the very complex interdisciplinary connections in orchideology and the enormous spectrum of contents of publications about orchids.

An electronic library or literature database may include a very wide selection of publications from all the above mentioned fields, not only about orchids but about botany in general. To use a metaphor: such a database would be very broad but not deep, it would be a rather flat disc with a large diameter. The lowest level in such a database would be the family *Orchidaceae*. Such a database would produce a large number of general information, but it would be very inefficient for an orchid specialist because a search for a combination of keywords like „molecular biology" **or** *Orchidaceae* would end up in a vast and confusing number of citations. The search for the combination „molecular biology" **and** *Orchidaceae* on the other side would end up in a small but incomplete number of citations. The contrary of such a „general"-database is a „special"-database, in this case also it would contain publications from all the above mentioned fields but only such documents in connection with *Orchidaceae*. To use the same metaphor: this database would be like a cylinder, very deep but with a limited diameter. Such a database is build especially for users interested in *Orchidaceae*, for other users the result of a search would be to specific.

The structure of a computer-based bibliography is variable in detail but the overall principle is always the same: the possibility to search for literature based on different criterions. The question whether the entire publication, the summary of a particular publication or simply the citation together with keywords are available in a database, depends only on the availability of the literature itself and the situation concerning copyrights. Older literature is already in the public domain, the question is whether the effort to scan such publications is proportional to number of accesses by the users of the database. To scan a very old book will be difficult by any means and the effort is high, it would not make sense to spend time and money for a very few interested users only. The size of a computer-based database is basically a function of two parameters: first the human resources – time and financial support – of the institution which is maintaining the database, and second the availability of publications fitting in the frame-work of the database. Especially the financial point is the limiting factor for size and completeness of an electronic database. The more specialized this database should be and therefore the less potential users one may expect, the more difficulties an institution will have to obtain the financial resources. This criterion is - at least for the time being - independent from the available technology. The question about the completeness of a computer-based literature database in science is therefore easy to answer: the narrower the definition of the content the higher the degree of completeness, the broader the definition, the less complete the database will be.

Another criterion to judge the quality of a database is the strict neutrality concerning the importance and quality of the included publications or documents. It is a common place that those who are maintaining a database will have almost certainly their own ideas about importance and quality of the content of publications they include. This idea is also almost certain different from the opinion of the users. It is therefore paramount to avoid any valuation of publications, if such documents fit in the definition of the database, they have to be included. It is strictly up to the user to make an own selection and judgment of the content of a given document. A third criterion is the consistency of a given database, it is important that the internal organization of a database is consistent and that also the procedure of adding new documents guarantees consistency. In other words, a given document should have the same keywords and the same form, independent when the entry was included in the database and by whom.

In order to understand what system and philosophy

a database should follow, we need to understand the needs and wishes of the user. Everybody who was sometime or other in the position to do literature research for an own project or publication, knows exactly how time consuming and often expensive this task can be. Such a process can be divided in two very clearly separated phases. The first step is the providing of a list of publications or citations in connection with the target of the planned work, the second step is to obtain the important publications in full insofar as they are really needed.

The first phase often is extremely time-consuming because such information has to be compiled from different physical sources or electronic databases. With exception of BIBLIORCHIDEA there is no other comprehensive database or bibliography of literature exclusively about or in connection with orchids in all aspects existing. An example: the Index Kewensis covers all taxonomic first descriptions of plants including the members of the orchid family, but there is no information about available monographs or revisions of a given orchid genus. Dissertations are covered by specialized databases of their own, and information about publications from interdisciplinary areas like molecular biology are not or only in parts included in databases about botany. The search for literature will therefore end up in a more or less long excursion through different computer based databases or printed sources. Unfortunately most of those databases will have a different system, a different user interface and different search engines.

The second phase, the acquisition of the „real" literature based on several lists of citations, is also often rather difficult and time consuming. Usually public libraries or university libraries are delivering on request exactly what the customer is ordering, nothing more and nothing less. If the citation is wrong or incomplete the customer will get wrong or incomplete response, occasionally the library will ask for more detailed information. The mere number of definitely wrong literature citations in publications is amazing and frightening; the range spreads from invalid or incomplete abbreviations to wrong volumes, wrong authors and wrong page numbers. Obviously the process of search for literature has become so time consuming and expensive that in order to save time authors are copying citations from other sources without ever have seen the literature itself. It is an open question whether this is a scientifically acceptable way to work, but the example shows the problem for a scientist to collect the necessary literature for a given project in acceptable time. Even if a good literature collection is at hand, the problem is not solved, the search for certain things and without clear citations in available literature is time consuming too. It seems to be clear that today no library can employ an orchidist in order to handle orders for orchid literature, and there are many other plant families with exactly the same problem. It is also clear that library staff cannot spend time to check in detail all unclear or incomplete orders from customers, there is but limited time available, if it cannot be spent the order goes back to the customer with a respective remark. There is one consequence out of all these facts: search for literature in an acceptable time and with acceptable effort can be done only by using a specialized database with a library in the background in which we find physically all the documents or publications included in the database and with a staff who is specialized in this area. The combination of library, database and specialized staff is paramount.

For the people maintaining the database it is important to know what the potential user needs. Also in this case the spectrum is very broad, from a simple search for the correct spelling of a certain epithet to a search for literature as basis for a monograph or dissertation almost everything is possible. The structure of the database should ensure that questions from allover this area can be answered. Because no database is complete, it cannot be expected to get a complete list of publications about a certain issue, but the list has to be complete enough for a start.

BIBLIORCHIDEA

Based on the fact, that the time available for literature search is limited, the project BIBLIORCHIDEA has been developed over a period of about 18 years. The inner structure of this computer based bibliography and the story of its development is a good example to show how a „special" database is build up. At the beginning it was a very simple structured list of available books and periodicals in an already rather large private orchid library, the main target was to ensure that the same book was not purchased or ordered twice.

There was no connection of the entries by selected keywords, the target was only to get fast information whether a certain publication was available or not.

In a second step, this list was integrated in professional and special software for library management. At this time the used software LIDOS was one of the most sophisticated and powerful tools available in this area. Very soon it became clear that the capacity of the used software would allow a much better and finer definition of the integrated entries or documents, and with this a much deeper and further use would be possible. It would result in much more than just a list of publications available from a library. So all the necessary information to order or to find a given publication has been added as well as information about author, co-author, year of publication, title, editor and publisher. Another point was the selection of the used standards on which citations would be based. For plant names Index Kewensis is the standard, for single publications (books) it is STAFLEU's Taxonomic Literature, for periodicals and journals it is BPH and BPH-supplement and for everything in connection with herbariums it is Index Herbariorum.

In a next step a keyword catalogue was build up, this collection of keywords would allow a search for literature after its content. Right from the beginning a hierarchically organized structure was chosen, which would make it possible to select the appropriate degree of selectivity for each search. The difference between a search for all publications about orchids in Europe, or orchids in Switzerland or orchids in the area of Zuerich is quite obvious. For Europe as keyword we would end up with virtually thousands of documents, for Switzerland still with hundreds and for Zuerich probabely only with some twenty documents. This hierarchical structure allows the user a very specific search with a manageable and clear number of answers or documents. Today there are six different levels in the keyword catalogue, an example will show this:

1th level (main keyword)	Geography
2th level	North America
3th level	USA
4th level	Florida
5th level	Everglades

Very soon it became clear that with this structure it would not be possible to integrate periodicals or journals. Hence in a next step all entries concerning periodicals have been removed and replaced by the article in the periodical itself. In order to integrate the correct citation of a particular article a new submenu or field in the entry menu had to be created and the title of the periodical was consequently integrated in the keyword catalogue. With this a search for a particular article and the search for all articles in a particular journal has become possible. This step of integrating articles was connected with enormous effort. Between 1841 and 1902 the well-known journal Gardeners' Chronicle alone contained not less than some 12'000 articles about or in connection with orchids, the reports about the sessions of the Orchid Committee of the Royal Horticultural Society not included. Today BBIBLIORCHIDEA contains about 120'000 articles from about 1'400 different journals and periodicals. Some of these journals are integrated completely, that means from volume one up to the recent number or volume with all articles (e.g. Orquidea (Mex) and Orquidologia), of others all articles in connection with orchids are integrated (e.g. Selbyana, Botanical Leaflets Harvard University) and of some only the known articles about orchids are integrated (e.g. American Journal of Botany). The main problem here was access to the primary literature, some of these journals are rather difficult to obtain because they are old or because they are not very widely circulated in libraries. Together with the titles of the journals the keyword catalogue increased to a number of some 25'000 keywords.

In the same time another submenu or field in the entry menu was introduced, in the field „species and below" we find an alphabetical list of all new taxa below generic level described in the particular publication or document. This part is in fact something similar to the Index Kewensis but the information is neutralized. That means the information is not whether a certain new taxa is valid or not according to the rules of botanical nomenclature or whether it is a synonym of something else, the information just states that the particular author had described this particular taxa in this particular publication. Again, it is up to the user to evaluate the information. Right from the beginning also varieties, formae and subspecies have been included; these names are not included in the older volumes of the Index Kewensis. In the meantime

about 65'000 taxa below generic level are included in Bilbiorchidea, with this an estimated rate of about 80 % of all described taxa below generic level in the orchid family is already accessible. Naturally also all taxonomic first descriptions of entities between species level and subfamily (e.g. genus, subtribe, tribe, hybrid genus) are included in the database, these entities are integrated in the field „above species".

The fast growing keyword catalogue showed that in some cases an explanation of the keyword was neccessary. Especially in some categories of keywords like titles of periodicals or individual personal names, an explanation of the keyword becomes important. The reason for this lie in the fact that by definition keywords have to be short and clear - no sentences - and that, wherever abbreviations are used, the complete, not an abbreviated form has to be available also. The example of the BACKHOUSE dynasty in England shows very clear why such explanations are important. The names of all three members of the BACKHOUSE dynasty are included as keywords, all three have the name James BACKHOUSE, the only difference is the date of birth and death. To avoid mistakes, the commentary to those three keywords explain exactly which James BACKHOUSE was father, son or grandson. These commentaries are accessible through the keyword catalogue. Especially important are the commentaries in the categories hybrid genera (parents and valid RHS-abbreviations), individuals (personal data), book series and periodicals (abbreviations after BPH and information about changed titles). The commentary of the title of a given periodical contains the full title of the journal, the official abbreviation after BPH, information about the time and extent, and information about a possible succeeding and preceding title of the journal, again with full changed title, BPH-abbreviation and extent. With this information a journal is defined in a very clear way, which is important considering the fact that rather often titles of journals are quite similar (e.g. Orchids (AOS), Orchids (South Africa) and Orchids (Australia)).

Another change was the decision to integrate iconographies like the Lindenia or Icones Plantarum Tropicarum not as a complete and single document, but by plates, that means every single plate was treated as a document of its own. With this decision again the number of entries or documents was increasing dramatically, the second edition of the field guide of the Orchids of Venezuela, published in 2000, alone added some 1'100 new documents to the bibliography. Together with this increase also the content of information increased, it was now possible to search at the level of single species and to find very fast an illustration of a particular plant. The plate by plate introduction of complex publications like the above mentioned Orchids of Venezuela and Icones Platarum Tropicarum, or like Flora Brasilica, Flora Brasiliensis, Venezuelan Orchids Illustrated and many others, was completed after about one year. The result of this task is the possibility to gain much more detailed literature citations.

The very fast development in computer technology and also the availability of better and more sophisticated software in connection with the fast growing importance of the internet were responsible for another decision about the future of BIBLIORCHIDEA. The existing database in its original DOS-based interface was available for interested users for several years under the name LITBUL. In order to keep BIBLIORCHIDEA up to date concerning the large number of new publications and because the structure especially of the keyword catalogue was changed and enlarged from time to time, a simple upgrade for the user was not possible. The only solution was a complete renewal of BIBLIORCHIDEA at least once a year. With this interval the database was in the worst case about one year behind. The process of creating renewals was expensive and not very efficient. Since the value of such a database is measured also on its being up-to-date, it was very important to find a way to maintain it in "real-time" via internet. Beside this, the used DOS-based software was old, it was not possible to get printouts of a search result in an easy way without data-transfers into word processing programs and it was not possible to use the mouse. This overall unsatisfying situation could be changed only by a fundamental change of the software environment. Consequently the decision was taken to extract all the data from the old software and to put them in a totally new software environment and, consequently make the new form accessible through the internet. Today new entries, corrections or changes in the data are done directly via internet, as a result of this on-time maintenance, BIBLIORCHIDEA is up-to-date all the time.

Naturally also BIBLIORCHIDEA is not complete

and there is much doubt whether it ever will reach completeness, but it will grow continuously. Some orchid journals like Orchid Review or American Orchid Society Bulletin are not yet completely integrated and from other periodicals we still are looking for missing volumes. On the other hand, many periodicals are already in the library but not yet included in BIBLIORCHIDEA. The limiting factor is not only the time, it is also the availability of the literature; in order to add new entries or documents, we need a physical copy of the original publication, without this we would copy old mistakes and we could not define the keywords. For the next five years we will have about 10'000 new documents per year, of these about 2'500 will be new publications and about 7'500 old publications which have become available in the meantime. With about 150'000 entries we will reach a platform, the increase per year will then be reduced to about 2'500 new publications and about 500 old ones. Probably we will then have the time to reprocess some documents with the goal of an further and finer classification. Especially some of the fundamental works about orchids, like SCHLECHTER's publications in Feddes Repertorium, we would like to divide in smaller parts. Today BIBLIORCHIDEA contains about 140'000 documents, included in this number are articles from all kind of periodicals, books or „single publications", catalogues, dissertations, checklists, manuscripts and iconographies. All these documents, as far as they have been published, are included in a form with enough information to order them through a public or scientific library. All of them are also represented in our library as physical copies. One of the hopes for the future is that authors all around the world would realize that the best way to make their own publications known would be to send us a copy in order to add it to BIBLIORCHIDEA as fast as possible. This is especially important for publications which are not widely distributed, like dissertations.

The actual form of BIBLIORCHIDEA as it is accessible through internet (www.Bibliorchidea.org), will allow the user different kinds of search or also the connection of different search methods. These are

- Direct search for author and co-author
- Direct search for the year of publication (selection direct or in a time-window)
- Search with text-input in the fields Title, Literature quotation, Editor, Publisher, Above species and Species and below (free-text search)
- Direct search for new descriptions in the respective fields Above species and Species and below
- Search for keywords by direct selection from the keyword catalogue as single keyword or in connection with other keywords by using the connecting terms and / or (Boolean connections)
- Enlarge the result by using one of the above mentioned methods.
- Restrict (decrease) the result by using one of the above mentioned methods (decrease to and Decrease by – functions)

Besides the search mechanisms, the software naturally allows the sorting of results by different criterions and the printout as list of documents or as single document with all the detailed information. The important addition is the fact that all documents the user can find in BIBLIORCHIDEA are also available as physical copy, hence a very fast access is guaranteed.

According to the very fast technological development especially in the information technology, it is extremely difficult to guess what development a database like BIBLIORCHIDEA will see in the next years. Certainly BIBLIORCHIDEA will remain a most important tool for everybody who need orchid literature for profession or hobby.

Rudolf Jenny is chemist by training and director of a company active in the industrial application of ozone. He is interested in the taxonomy, pollination ecology and history of orchids, especially in the subtribes Catasetinae, Huntleyinae and Stanhopeinae. He is author of treatments of several genera in those subtribes, especially Stanhopea and Gongora. He owns one of the most complete orchid libraries iiin private hands and maintains the literature database BIBLIORCHIDEA. Beside this he is regularly publishing about orchids in different periodicals.

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A FORM AND CHECKLIST FOR THE DESCRIPTION OF ORCHIDS IN THE FIELD AND LABORATORY WORK

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RESUMEN. Se creó un formulario para la toma de datos durante la descripción de orquídeas en el campo y en el laboratorio. Éste contempla las caracteristicas más importantes que deben ser anotadas para una posterior identificación de las especies con el uso de claves dicotómicas. Además, incluye listas de los téminos botánicos más comunes utilizados en la descripción de plantas y flores. Su utilidad es muy grande, tanto para afisionados como profesionales, para facilitar la toma de datos y para asegurar que ésta sea lo más completa y sistemática posible. El formulario está disponible en formato pdf en www.bosquedepaz.com.

KEY WORDS: orchid description, data collection, field notes, form, descriptive terms, Bosque de Paz.

Just as amateur bird watchers often provide useful information to professional ornithologists, amateur orchid enthusiasts can make valuable descriptions of orchid plants and flowers observed in the field or in private collections. These observations, with the aid of glossaries, keys, field guides, photographs, illustrations, and herbarium material, could eventually lead to a positive identification of the plants down to the species level. Observations of this kind can play a key role in fulfilling the need for careful inventories of orchids in natural forests. These inventories can form a baseline for research regarding the effects of informal, *i.e.*, illegal, collections from the wild, the impact caused by habitat loss, and as supplemental material for biogeography studies and other research applications in orchid ecology.

However, such detailed information about a species is seldom available for most plant preserved in herbaria around the world. Herbarium labels do include a short description of the plant, but these descriptions are, more often than not, vague and ambiguous, and may refer more to the conditions of the site where the plant was collected, rather than specific the morphological and anatomical characteristics of the plant itself. More detailed descriptions of plant parts can be found in the field or laboratory notes of the scientists who handle the specimens, but as is the case with the herbarium labels, these descriptions are usually unavailable to the general public. Therefore, the terminology associated with orchid taxonomy, and even more, the structures that give the most information about a particular species, may be poorly known to the untrained enthusiast, and this can make orchid identification and appreciation hard for the beginners, and even harder for the experts who make an effort to identify and categorize all the informal information provided by the amateurs.

T The process of becoming familiar with botanical terms, and particularly the vocabulary regarding orchid taxonomy, can be a daunting task for amateurs who lack any background formation and training in botany, or even in general biology. This will often cause them to overlook basic plant and flower structures when observing orchids in the field. Furthermore, omissions of this kind can later diminish the chance for positive identification of the plants down to the species level, because they create ambiguities and misinterpretations of the somewhat technical identification keys. In an attempt to reduce inconsistencies, a simple fill-out-form to record these features has been developed. This form is intended to have a clear and intuitive structure, which allows for an easy search of specific features, and includes lists of many of the taxonomic terms that are used to describe each of the particular characters presented. Given that these lists are not meant to be comprehensive, i.e., are included as a vocabulary aid for the inexperienced user, previous study of the technical terminology used for orchid identification is advised. Any book or glossary of general botanical terms can

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Checklist for Recording the Description of Orchids

Field number _____ Collected by: _____ Date:_____ month/day/yr

Location: _____ Geographic Locality _____ Latitude, ° Longitude, °

Identification _____ Genus _____ species _____ References

Described by: _____ *Identified by:* _____

Plant form: _____ **Plant size** (growth length), cm_____ **Growth**_____ Monopodial or sympodial

(erect, creeping, arching, fan, clumped, canes, distichous, equitant)

Root notes (thickness, succulent?, etc.) _____

Pseudobulbs (PB) _____ present? (Y/N) _____ internode number _____ axial shape _____ section shape (compressed? ribbed?) _____ sheathing bracts?

Dimensions, give range, mm _____ height _____ width _____ thickness _____ PB separation, mm

Leaves Number per pseudobulb _____ (where variable, give range)

Number of leaves per growth: _____

Plan shape _____ subulate, linear, oblong, lancolate, oblancolate, ovate, obovate, elliptate, spatulate, deltoid, reniform, chordate, triangular, trullate, sagittate, hastate, lingulate

Transverse shape _____ conduplicate, pleated, terete, semiterete, flat, keeled, rolled, furrowed,

Leaf tips _____ acute, acuminate, bifid, indent, obtuse, retuse, emarginated, awn, praemorse, tridentate, caudate, truncate

Leaf margins _____ (smooth, crenate, serrate, etc.)

Dimensions, mm _____ length _____ width _____ thickness _____ surface texture _____ surface color/pattern

Flowers

Inflorescence

Total stem length, mm____Bract/spathe notes _____ Determinant (Y/N)?_____

Origin _____ axillary, basal, terminal, leaf/petiole join (leaf base), axillary leaf opposed

Type _____ single-flowered, spike, panicle (spray), raceme, cymose, umbel, or fascicled _____ No. Flowers

Pedicel _____ Length, mm _____ Thickness, mm _____ Notes (shape, etc.)

More flower notes:_____

Perianth Flower dimensions, mm: _____ Width _____ Height _____ Front to Back

	Color	Shape	L X W, mm	Color Pattern	Margins	Notes
Dorsal sepal						
L. sepals						
Petals						
Labellum						

_____ vestiture (callus, hairs, papillae, ridges, …) _____ resupinate?(Y/N) _____ spurs, or nectaries? _____ fragrance?

Lip notes: _____

Column

_____ Color _____ Shape _____ L X W, mm _____ Margins _____ Anter cap

_____ Mentum? _____ Vestiture _____ Pollinia no. _____ Pollinia attach. (caudicle/stipe) _____ Notes

Column notes: _____

Ovary _____ L X W, mm _____ Shape

Seed pod _____ L X W, mm _____ Shape _____ Seed pod and seed notes

Collection notes: _____ Pressed plant? _____ Pressed flowers? _____ Pickled flowers?

Photography notes: _____ Plant? _____ Exposure ID _____ Flower detail? _____ Exposure ID _____ Dissected flower? _____ Exposure ID

See drawings illustrating descriptive terms in Bechtel *et al.* 1992, Sheehan & Sheehan 1994, Hudgson *et al.* 1991 and Stewart 1995.

Created by Stephen Kirby, Ph.D.and Melania Muñoz, Version 1.4, January 2007, Bosque de Paz, Costa Rica. Acknowledgement to Piero Protti for his help in the elaboration and advice of this form.

FIGURE 1. The English version of the form for recording field and laboratory data describing orchids.

prove useful, but more specific orchid references such as Hudgson *et al.* (1991), Bechtel *et al.* (1992), Sheehan & Sheehan (1994) and Stewart (1995), are highly recommended. Measurements of the dimensions of most of these structures may be recorded in the appropriate blank spaces when considered necessary. Space is also provided for some detailed descriptive notes, and wide margins allow extra space for sketches or illustrations, if required.

This form was initially used for the recording and description of more than 160 orchid species collected at Bosque de Paz Biological Preserve (Alajuela, Costa Rica) in June 2004 (Muñoz and Kirby, this volume). Since then, both the form and checklist have been continuously improved from experience with use, and have also been translated into Spanish. The collected data have been used for formal plant identification later on, down to the species level when possible. This is the reason why the authors consider that it could also be useful to other researchers and orchid enthusiasts, not only for field collection, but also for laboratory descriptions, because it can facilitate data collection, and ensure that it is as complete and systematic as possible. Furthermore, that information can be filed in a more organized manner than how it is currently done as field notes and/or herbarium labels. It can also be converted to an electronic format and be used with a PDA (Personal Digital Assistant) or a laptop. Information could be recorded and immediately stored electronically in the field, a laboratory, or at home.

The form has been designed to fit on both sides of a single U.S. letter sized sheet of paper (8? in. x 11 in.), but may easily be adapted to A4 or other larger paper sizes. A pdf file of this form is available free of charge both in English and in Spanish, as an attached file at www.bosquedepaz.com. It can also be e-mailed upon request of the authors. The English version of the form is showed in figure 1.

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LITERATURE CITED

Bechtel, H., P. Cribb, & E. Launert. 1992. The Manual of Cultivated Orchid Species. 3rd Edition. MIT Press, Cambridge, Mass. p. 568-572, 585 p.

Hodgson, M., R. Paine, & N. Anderson. 1991. Letts Guide to Orchids of the World. Letts Publishing, p. 2-14 and p. 229-232, 236 p.

Muñoz, M. & S.H. Kirby. 2007. An orchid inventory and conservation project at Bosque de Paz Biological Reserve, Upper Río Toro Valley, Alajuela, Costa Rica. Proceedings of the 3rd International Orchid Conservation Congress *in* Lankesteriana, this volume.

Sheehan, T. & M. Sheehan. 1994. An Illustrated Survey of Orchid Genera. Timber Press, p. 381-412, 421 p.

Stewart, J. & M. Griffiths (eds.) 1995. Manual of Orchids, 388 p. Royal Horticultural Society, Timber Press, p. xxxv-liii.

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EPIDENDRA, THE BOTANICAL DATABASES OF JARDÍN BOTÁNICO LANKESTER AT THE UNIVERSITY OF COSTA RICA

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Introduction

It may seem somehow out of line to present a new system of botanical databases in the context of a meeting on orchid conservation, for two main reasons. Even though botanists have been rather slow in upgrading to the use of electronic databases (with some early controversy regarding the desirability of the application of electronic data processing methods to taxonomic problems as a whole, see *i.e.* Shetler 1974), the dissemination of plant information via the web has grown steadily in recent years. So, why *another* system for electronic retrieval of botanical information? On the other hand, the role of natural history collections data is perhaps better defined today as for its two-fold relevance in research and education than with respect to the practicality of information in conservation efforts. Can a system for electronic interchange of plant information be of real use as a conservation tool?

I hope that trying to answer these two questions may explain the reasons for creating EPIDENDRA, the botanical databases system of Jardín Botánico Lankester (JBL) at the University of Costa Rica, as well as illustrate some useful characteristics of this project.

Access to the sources

For centuries, scientists have amassed information on plant life, describing and naming more than a quarter million of species on the planet. When organized in the format of floras, information included relevant data not only about morphology, but also on distribution and other aspects of plant biology. It is true that from the personal computer in his office, in any part of the world, a botanist may instantly link today to a number of powerful electronic databases, avoiding the time to correspond and to travel to botanical libraries and herbaria in order to gather the requested information, an activity that only a few decades ago would have taken months (Allen 1993). However, it may be useful to understand which kind of information is mostly available in actual databases, and how we can improve information access.

If one accesses today the TROPICOS database, launched in 1983 by the Missouri Botanical Garden (which has been a leading institution in computerizing plant information), he can find a system dealing with tens of thousand of plant names from around the world, in many cases cross-referenced with distribution maps and other non-taxonomic information. The system is designed to provide references to plant names, basionyms and synonyms, nomenclatural types, and lists of *exsiccata* for selected regions, allowing botanists to gain ready access to the authors of names, the titles of key publications and, indirectly, to the location of type specimens. This system of references has shown its relevance in floristic projects as the Flora of North America, the Floras of Panama and Mesoamerica, the Flora of Peru and the Flora of China, and it provides daily information for researchers working with tropical floras around the world, including the staff of JBL.

To restrict the field to orchids, the database BIBLIORCHIDEA, now hosted by the Swiss Orchid Foundation and operating under patronage of the University of Basel, represents the largest orchid literature database worldwide, containing most of the existing journal articles, books and preprints on orchids with over 140,000 entries. The database offers a nearly complete system of references to the titles of publications, extending the coverage not only to the original protologues but also to different types of literature quotations (for more details, see Jenny 2007). Numerous, less "institutional" databases, mainly aimed to quick orchid identification via

electronic images, exist on the web, but the quality of the provided information is often not totally reliable, and they will not be considered for the purpose of this work.

One common character of the available tools for electronic retrieval of botanical information is that they provide a system of references, which supposes some facility in the direct access to the sources through libraries and herbaria services. This is often not the case in tropical countries, where facilities are insufficient, if not absent, and where the lack of historical libraries and the relatively "modern" herbaria represent a major obstacle for botanic research when concerned with the retrieval of historical information (Gómez-Pompa & Nevling 1988, Pupulin & Warner 2005).

Some steps in this direction have been made in recent years, through the digitalization of type specimens in several institutions. Noteworthy is the recently completed project of digitalization of the Oakes Ames Orchid Herbarium types at Harvard University. However, it is perhaps interesting to note that the first actions of this project were done in the framework of a cooperative effort between the Harvard University Herbaria and the University of Costa Rica, originally aimed to the digital imaging documentation of the types of Costa Rican Orchidaceae (Pupulin & Romero 2003).

One of the more crucial points to be resolved in order to achieve the goal of an open system for the retrieval of biological sources is the sociological impediment to data interchange, through the protection of copyrights and intellectual property, concerning ownership and ultimate usage of the information. Most of the valuable documents relative to the tropical flora are stored in institutions of the developed countries, sometimes jealous of the historical value of the owned sources. It is curious to note, as Conn (2003) did, that copyrights concerns are vigorously debated when the source collections are presented in a digital format, but not when available as physical collections *per se*. However, the recent agreements signed by the University of Costa Rica with the Harvard University Herbaria and with the Herbarium of the Royal Botanic Gardens, Kew, to digitally document the specimens and the associated data of the orchids from the Mesoamerican region, are an unquestionable step in the right direction.

Conservation data

Natural history collections have always contained a large amount of data providing biogeographic, ecological and biographical information through the labels affixed to the specimens, and they have been considered an indispensable resource for conservation policies, documenting what we do and do not know about the biota (Lane 1996). Nevertheless, while the threatened tropical biota is the major biological concerns of today's humankind, and the need for floristic research in the tropics is greater that in any other time in modern history, most of the global important collections are stored in developed countries. This has been an impediment to a vaster documentation of biological variation, which is required for a full understanding of living diversity, ecosystem dynamics and their conservation. Our question should be if the actual documentation of tropical biodiversity (or orchid biodiversity, to restrict to our concerned topic) is sufficient to help the conservation "movement", transforming floristic research into an actor in the conservation play. The actual figures point toward a negative answer. In a short review of the available records kept in six major herbaria relatively to 350 Costa Rican orchid species, Dressler (1996) found that 78% of the taxa were represented by less than 6 collections. Of those, 20% were based on a single collection, and for 74 species (21%) he can not find a single herbarium specimen in the herbaria sampled. The obvious incongruity is that we do not know the flora of the tropics enough to really orient conservation policies, mainly if we consider that only at most 15 percent of the life diversity on Earth has been apprehended by science, and new species are turning up constantly from the scattered expeditions to rich tropical areas.

The possibility to rapidly document the presence of some species in a given area via the access to reliable electronic data may be essential in influencing decision makers at any level, but once more the quality and efficiency of this documentation is directly associated to the amount of the available information. This quality must be increased not only by a continuous updatement of distribution records, but also providing more efficient identification and "emotive" aids, like visual databases of specimens, slides, drawings, etc., helping to match the specimen with known taxa. According to

Flecker (2000), the administration of Harvard University granted 12 million dollars to the University Library for a 5-year project aimed to build a digital library infrastructure. However, this is often not the case where funds for research and documentation are limited (as in developing countries), and the justification of scientific activity through the provision of services to the general public is probably critical.

EPIDENDRA

The past debate on biological databases has mainly focused on the best model to be used in organizing the taxonomic data from literature and other sources to avoid over-simplification and to reflect the elasticity of taxonomy as well as alternative taxonomies (see, i.e., Berendsohn 1997, Conn 2003). Even though the "unofficial" adoption of one or more of the alternative taxonomies can not be avoided in the daily work, taxonomic information may become outdated very rapidly in the tropics, and this perhaps tends to reduce taxonomic decisions in the database system to a minimum. The only alternative would be to build a system and a trained staff which avoid mistakes in the capturing and management of the information, but this would greatly increase the cost of the effort.

The main constraints to the creation and maintenance of biological databases in tropical countries have been reviewed by Gómez-Pompa and Nevling (1988) and I refer to their paper for a critical analysis. It is unfortunate to say that, with the exception of computing technology, most of these constraints have not found positive solutions. However, botanists working in tropical areas have an immense opportunity to improve our knowledge of life diversity and to provide a bridge between systematic research and the general public, incorporating to their source-based systems other data which are not accessible to their colleagues in the first world. They include field observations on species frequency and natural variation, susceptible habitats, pollination biology, relationships with other organisms, etc. But, foremost, tropical botanists have the still unachieved chance to "portray" biodiversity for the use of the public through *in studio* work, mainly based on digital imaging. Knowing something always makes it more valuable, and only what it is valued will ultimately be saved.

LITERATURE CITED

Allen, W.H. 1993. The rise of the botanical database. Biosc. 43: 274-279.

Berendsohn, W.G. 1997. A taxonomic information model for botanical databases: the IOPI Model. Taxon 46: 283-309.

Conn, B.J. 2003. Information standards in botanical databases – the limits to data interchange. Telopea 10: 5360.

Dressler, R.L. 1996. Costa Rica and its orchid diversity. Proc. World Orchid Conf., Rio de Janeiro: 321-331.

Flecker, D. 2000. Building a first generation digital library infrastructure. D-Lib Mag. 6, consulted at http://hul.harvard.edu/ldi/.

Gómez-Pompa, A. & L.I. Nevling Jr. 1998. Some reflections on floristic databases. Taxon 37: 764-775.

Jenny, R. 2007. What is BIBLIORCHIDEA? Proceedings of the 3rd International Orchid Conservation Congress *in* Lankesteriana, this volume.

Lane, M.A. 1996. Roles of natural history collections. Ann. Missouri Bot. Gard. 83: 536-545.

Pupulin, F. & G.A. Romero. 2003. Costa Rican Orchidaceae types (Crotypes) digital imaging documentation at AMES, Harvard University. Lankesteriana no. 7: 11-16.

Pupulin, F. & J. Warner. Know your orchids. A botanical garden rich in information serving a country rich in flora. Orch. Res. Newsletter 6: 5-8.

Shetler, S.G. 1974. Demythologizing biological data banking. Taxon 23: 71-100.

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EFFECT OF KNOWLEDGE GAIN ON SPECIES CONSERVATION STATUS

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Introduction

Following the 2002 World Summit in Johannesburg, the Convention of Biological Diversity (CBD) called for a decrease in the rate of biodiversity loss by 2010 (www.biodiv.org/2010-target). However, a 2003 UK Royal Society report on "Measuring Biodiversity for Conservation" discussed the lack of satisfactory measures of biodiversity, and the difficulty in accurately reporting the loss of biodiversity by 2010. Even more pressing is the need to obtain reliable measures of extinction risk in order to prioritise proposals to reduce the rate of biodiversity loss. The World Conservation Union (IUCN) defined a set of categories for conservation status supported by decision rules for assigning species to these categories (IUCN 2001). These rules have received international acceptance and have become one of the most important set of tools for making decisions in conservation biology. However, assigning species to one of these categories often requires large amounts of data and extensive fieldwork. For most species these data are not available, and are not easily obtained; often the only available data are a "handful" of sighting records, both from the field and as records in specimen-based collections (Solow & Roberts 2003). This is related to the level of uncertainty involved and applies to the prediction of future events, to physical measurements already made, or to the unknown.

It is estimated that there are around 2.5 billion specimens in biological collections. However, the potential contribution of natural history collections has gone largely unnoticed by the public and policymakers (Suarez & Tsutsui 2004). These records provide information on the distribution of taxa through time and space, and represent primary, verifiable observations. The value of this information is growing with the demand for rapid and inexpensive conservation assessments (Shaffer *et al*. 1998, Willis *et al*. 2003). In addition, demand is also growing for the data to be provided over the internet (e.g. www.gbif.org) as part of the CBD's policy of open access and benefit sharing.

There is therefore a need for the development of statistically rigorous methods for the production of conservation assessments from limited data, particularly those found in biological collections. Several methods have been developed which provide a probabilistic basis for an extinction hypothesis based on such sighting records (Solow 1993a, 1993b, Roberts & Solow 2003, Solow & Roberts 2003, 2006; McInerny *et al*. 2006, Solow 2006, Solow *et al*. 2006a, 2006b). These methods depend on sighting records of a species arranged as an order statistic ($t_1 < t_2 < \ldots < t_n$) within some time period, T. To make use of these methods it is necessary to have an understanding of the collection process itself. Therefore, any attempt to use biological collections to draw inferences about species conservation needs an understanding of the collection process itself (McInerny *et al*. 2006, Solow & Roberts 2006, Roberts & Solow submitted). Care must therefore be taken to avoid bias due to sampling effects when inferring the conservation status of a species. This bias can vary considerably between taxa and geographical regions, one such form of bias is through access to specimens because of CITES (Convention on International Trade in Endangered Species). For example, to meet the 2010 target conservation assessments are required, one possible method is to assess those species that collectively best represent global

diversity patterns. The family pairing of the Orchidaceae and Gramineae has been show to have the highest correlation coefficient with global genetic diversity (r_S = 0.973) (Nic Lughahda *et al*. 2005). However, given the bias, resulting from CITES regulations, on the accumulation and movement of Orchidaceae specimens (Roberts & Solow submitted), this may not be possible. Although CITES has enjoyed undeniable success, a long-standing concern in scientific circles, which has now been confirmed (see Roberts & Solow submitted), has been that CITES impedes the cross-border movement of scientific specimens. This concern is heightened as other international efforts to conserve biological diversity move forward i.e. Convention on Biological Diversity's and its implications for access and benefit sharing.

Another form of sampling bias can occur when comparing the distributions of two or more species based on collections containing different numbers of individuals. The relative paucity of specimens of some taxa may be related to the lag between the time of discovery and the time it takes for specimens to accumulate in collections. If sampling effort is consistently lower for recently identified taxa, there will be a tendency to underestimate key conservation parameters such as size ranges (Solow & Roberts 2006).

An Example

Solow and Roberts (2006) compared the number of locations two species of *Phragmipedium* from Ecuador are found at based on herbarium specimens. *P. longifolium* (Warsz. ex Rchb.f.) Rolfe has a wider distribution with 8 localities compared to *P. hirtzii* Dodson with 6. However, the distributions are based on a total of 18 specimens for *P. longifolium* whereas only 10 for *P. hirtzii*. This difference may be related to the fact that *P. longifolium* was described in 1852, 136 years before *P. hirtzii* which was only described in 1988. This means that there has been 136 more years to accumulate data on *P. longifolium*, such as the distribution, or rather a 136 year difference in sampling effort. If the sampling effort were the same, the expected number of locations for *P. longifolium* would be around 5.3 rather than 8.

Discussion

These considerations beg the larger questions as to why taxa are discovered when they are? And whether conservation and biodiversity prioritisation reflect a level of conspicuousness and accumulation of knowledge? Perhaps even more importantly for conservation biology is whether what we are recording is even representative of the underlying biodiversity? For instance do human tend to find large species more frequent than small species? Or do we see red-flowered species then say white? Answering these questions is particularly important given the time and money currently being spent on 'rapid biodiversity assessment'. Apart from a few papers on conspicuousness (Gaston & Blackburn 1994, Gaston *et al*. 1995a, 1995b, Allsop 1997, Collen *et al*. 2004), there has been very little work in these areas, and even less on the link between this area of research and conservation assessments.

Literature Cited

Allsop, P.G. 1997. Probability of describing an Australian Scarab beetle: influence of body size and distribution. J. Biogeogr. 24: 717-724.

Collen, B., A. Purvis & J.L. Gittleman. 2004. Biological correlates of description date in carnivores and primates. Global Ecol. Biogeogr. 13: 459-467.

Gaston, K.J. & T.M. Blackburn. 1994. Are newly described bird species small bodied? Biodiversity Letter 2: 16-20.

Gaston, K.J., T.M. Blackburn & N. Loder. 1995a. Which species are described first? The case of North American butterflies. Biodivers. Conserv. 4: 119-127.

Gaston, K.J., M.J. Scobe & A. Crook. 1995b. Patterns in species are description: a case study using the Geometridae (Lepidoptera). Biol. J. Linn. Soc. 555: 225-237.

IUCN (2001) Red List Categories and Criteria Version 3.1. IUCN Species Survival Commission, Gland, Switzerland.

McInerny, G.J., D.L. Roberts, A.J. Davy & P.J. Cribb. 2006. Significance of sighting rate when inferring extinction and threat. Conserv. Biol. 20: 562-567.

Nic Lughadha, E. *et al*. 2005. Measuring the fate of plant diversity: towards a foundation for future monitoring and opportunities for urgent action. Philos. T. Roy. Soc. B 360: 359-372.

Roberts, D.L. & A.R. Solow. 2003. When did the Dodo become extinct? Nature 426: 245.

Roberts, D.L. & A.R. Solow (submitted). The effects of CITES on scientific collections. Biol. Conserv.

Royal Society. 2003. Measuring Biodiversity for Conservation. Policy Document 11/03 (www.royalsoc.ac.uk/document.asp?tip=1&id=1365).

Shaffer, H.B., R.N. Fisher & C. Davidson. 1998. The role of natural history collections in documenting species decline. Trends Ecol. Evol. 13: 27-30.

Solow, A.R. 1993a. Inferring extinction from sighting data. Ecology 74: 962-964.

Solow, A.R. 1993b. Inferring extinction in a declining population. J. Math. Biol. 32: 79-82.

Solow, A.R. 2005. Inferring extinction from a sighting record. Math. Biosc. 195: 47-55

Solow, A.R., A.J. Kitchener, D.L. Roberts & J.D.S. Birks. 2006. Rediscovery of the Scottish polecat, *Mustela putorius*: Survival or reintroduction? Biol. Conserv. 128: 574-575.

Solow, A.R. & D.L. Roberts. 2003. A nonparametric test for extinction based on a sighting record. Ecology 84: 1329-1332.

Solow, A.R. & D.L. Roberts. 2006. Museum collections, species distribution, and rarefaction. Divers. Distrib. 12: 423-424.

Solow, A.R., D.L. Roberts & K.M. Robbirt. 2006. On the Pleistocene extinctions of Alaskan mammoths and horses. P. Natl. Acad. USA 103: 7351-7353.

Suarez, A.V. & N.D. Tsutsui. 2004. The value of museum collections for research and society. Bioscience 54: 66-74.

Willis, F., J. Moat & A. Paton. 2003. Defining a role for herbarium data in Red List assessments: a case study of *Plectranthus* from eastern and southern tropical Africa. Biodiver. Conserv. 12: 1537-1552.

David Roberts was educated at the Universities of Aberystwyth, Wales and Aberdeen, Scotland, where he received his doctoral on the "Reproductive Biology and Conservation of the Orchids of Mauritius". He is currently a senior scientific officer at the Royal Botanic Gardens, Kew, where he works on the orchids of West Africa, the western Indian and alternative uses of herbarium data, particularly relating to conservation. He will soon move to Harvard to take up the Sarah & Daniel Hrdy Fellowship in Conservation Biology, where he will be conducting research into the subject of this paper and lecturing.

New Technologies for Conservation and DNA Barcoding

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ALLOTETRAPLOID EVOLUTION IN *DACTYLORHIZA* (ORCHIDACEAE)

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KEY WORDS: *Dactylorhiza*, allopolyploidy, hybridization, ITS rDNA, gene conversion, plastid microsatellites

One of the most perplexing problems in Western European terrestrial orchid taxonomy has been how to deal with the large numbers of taxa that have been described for the allopolyploid taxa, which are the products of hybridization between taxa in the *Dactylorhiza maculata* (L.) Soó group and the *D. incarnata* (L.) Soó group (Table 1). The polyploidy hybrids are lumped under the category of *D. majalis* (Rchb.) P.F.Hunt & Summerh. s.l. (Table 1). By taking their ecology into account, it is clear that offspring from putatively the same parental taxa have different ecological preferences and been given taxonomic recognition as species by many authors. It has also been clear that within each of the parental complexes several distinct entities exist, again differing in their ecologies and morphology. Many of the named allotetraploid taxa are highly restricted, and therefore there are conservation implications if such taxa are foci of efforts to prevent them from disappearing. It is therefore appropriate to study such taxa, both on evolutionary and conservation bases.

To study these problems, we employed a genetic approach using two sets of markers, the nuclear ribosomal spacer regions (nrITS) and plastid microsatellites. These were first sequenced to determine if there were differences in length that could be used as characters, which was discovered to be the case. We then designed primers to amplify short fragments (140-200

TABLE 1. General taxonomy and distribution of Western European species of *Dactylorhiza*.

Ploidy	Species	Distribution
diploid	*D. foliosa*	Madeira
diploid	*D. fuchsii*	Western Europe, North Africa and Western and Central Asia; in the east replaced by *D. saccifera*
diploid	*D. saccifera*	Italy, Greece, the Balkans
diploid	*D. incarnata*	Western Europe, North Africa and Western and Central Asia
diploid	*D. euxina*	Near East
diploid	*D. sambucina*	Sweden to southern France east to Greece and Eastern Europe
diploid	*D. (Coeloglossum) viridis*	North Temperate Zone
auto-tetraploid	*D. maculata*	Western Europe, but difficult to separate from *D. fuchsii* in Central and Eastern Europe and rare in southern Europe
Allo-tetraploid*	*D. majalis* s.l. (including *alpestris*, *elata*, *occidentalis*, *praetermissa*, *purpurella*, *sphagnicola*, *traunsteineri* etc.)	Broadly distributed in Europe, Asia, and with a limited distribution in North America and Iceland ; some *s. s.* taxa with localized distribution (e. g. *occidentalis* only in western Ireland)

*Thought to have originated as crosses between the *D. incarnata* group and the *D. maculata* group, but exact parentage highly speculative and the major focus of this study.

base pairs) that contained the length-variable regions, and then these differences could be assessed by the length of the amplified fragments. This made the process quick. The short length of the amplified fragments also meant that it was possible to use DNA extracted from herbarium specimens; DNA from such sources was typically found to be highly degraded. Plastid DNA can demonstrate which of the parental taxa is the maternal parent of the hybrids. ITS rDNA is part of the nuclear genome and inherited biparentally, but hybrids soon begin a process of gene conversion and lose one of the two parental copies that they initially possessed. For fairly recently synthesized hybrids, both parental ITS alleles are present, but for older hybrids only one of these alleles remains. We have used these two sets of markers to dissect the complex patterns of morphology and ecology; the process of gene conversion in nrITS provides a relative timescale for the ages of the various groups of allopolyploids (Pillon *et al.* 2007).

It was relatively easy in western Europe to find genetic differences between the two most common species of spotted orchids, *D. fuchsii* (Druce) Soó and *D. maculata*. These species and the *D. incarnata* complex are also easily distinguished by both sets of markers. These are also ecologically and morphologically easily separated, but in eastern Europe *D. fuchsii* and *D. maculata* are difficult to distinguish on morphological grounds. All material labelled as *D. maculata* from Austria and Germany that we examined are recent hybrids; they exhibit nearly equal amounts of the two ITS alleles found in western Europe in *D. fuchsii* and *D. maculata*. We do not know the ploidy of these plants, but we suspect that they will turn out to be allotetraploids, as Shipunov *et al.* (2004) found in similar plants in northern Russia. These plants grow in acid sites, which in western Europe would contain *D. maculata*, and the morphology of these plants is intermediate between those of the two parents. We do not consider that *D. maculata* occurs in central and eastern part of Europe.

Allotetraploids that originated from crosses between *D. fuchsii* and *D. maculata* and members of the *D. incarnata* group are some of the most common and conspicuous orchids in Europe, and these too exhibit morphological and ecological differences. In addition to *D. incarnata* (almost always the paternal parent), one set of these was parented by an unknown diploid species, most likely found in southern Europe and similar to *D. foliosa* (Rchb.f.) Soó from Madeira and *D. maculata*. The correct name for these allotetraploids is *D. elata* (Poir.) Soó. These are older allotetraploids, and they almost always contained just the *D. maculata* allele without any remaining copies of *D. incarnata*. In Ireland, we found another allotetraploid, *D. occidentalis* (Pugsley) P.Delforge, that had exactly this same parentage, but these accessions contained both parental alleles, sometimes exhibiting conversion toward the *D. incarnata* allele. This, then, is a more recently formed allotetraploid than *D. elata*. Another set of allotetraploids was formed from a species similar to extant *D. fuchsii*, with older, *D. majalis* s.s., and younger, *D. traunsteineri* (Saut. ex Rchb.) Soó, forms.

Many authors consider *D. maculata* and *D. fuchsii* to be subspecies (of *D. maculata* s.l.), but nearly all authors agree that *D. foliosa* is distinct from *D. maculata* s.l. on morphological and ecological grounds. Although it is true that numerous hybrids occur between these two throughout their ranges (and in eastern and central portions of Europe this not pure *D. maculata*), this appears to be a recent phenomenon. None of the allotetraploids we examined exhibited mixtures of the *D. maculata* and *D. fuchsii* ITS alleles. These two entities are easily distinguished morphologically, and *D. fuchsii* is a calcicole whereas *D. maculata* is a calcifuge. Our results do not distinguish genetically between *D. foliosa* and *D. maculata*, so if authors wish to consider *D. maculata* and *D. fuchsii* as subspecies, then *D. foliosa* must also be considered as a subspecies of *D. maculata* s.l. Results from analysis of nuclear, low-copy chalcone synthase (L. Inda and M. Chase, unpubl.) indicate that the entity that gave rise to *D. elata* was closer to extant *D. foliosa* than to *D. maculata*. This finding points again to the fact that there must have been (or perhaps still is) a diploid species of the *D. maculata/foliosa* type somewhere in southern Europe and that *D. maculata* s.s. is not a parent of the *D. elata* allotetraploids that populate modern Europe. We have not yet examined the parentage of *D. occi-*

TABLE 2. Recommended framework classification of European members of the *D. incarnata* and *D. maculata* groups and their derived polyploid complex. The plastid haplotype and ITS allele(s) given here are considered typical of each taxon. This summary focuses on well-established species, incorporating regional endemics but excluding many local endemics.

Taxon	Ploidy and parentage	Plastid haplotype	ITS allele(s)
D. fuchsii (inc. *cornubiensis*, *okellyi*)	2X	A	V, IIIb
D. maculata (inc. *ericetorum*, *elodes*)	4X (autotetraploid)	B	I
D. saccifera	2X	C, G, W	VI
D. incarnata s.l. (all W European taxa)	2X	E	Xa
D. euxina	2X	Y, K	Xb
D. elata (North Africa)	*maculata* × *incarnata*	O	IIIa, completely converted
D. elata (Europe)	*maculata* × *incarnata*	B	I, most accessions completely converted
D. occidentalis (inc. *kerryensis*)	*maculata* × *incarnata*	B	I dominant, X in 1/3 or fewer copies
D. sphagnicola	*maculata* × *incarnata*	B	Xa dominant, I in 1/3 or fewer copies
D. majalis (inc. *alpestris*)	*fuchsii* × *incarnata*	A, C	V, IIIb, most accessions completely converted
D. praetermissa (inc. *junialis*)	*fuchsii/saccifera* × *incarnata*	A, C	V, IIIb, VI, often with VI dominant
D. traunsteineri (inc. *lapponica*)	*fuchsii* × *incarnata*	A, C	V, IIIb, rarely with Xa dominant
D. purpurella (inc. *cambrensis*)	*fuchsii* × *incarnata*	A	V, IIIb, rarely with Xa dominant

dentalis in the same detail as for *D. elata*, but this is underway. We present (Table 2) a provisional taxonomy for Western European *Dactylorhiza*, based on the results of this and other studies.

All across Europe, there are many sites where these species, diploids and tetraploids coexist, and in many cases researchers have been tempted to think that the hybrids arose locally. However, all of our evidence indicates that the hybrids arose elsewhere, further south in Europe, and migrated along with the diploid progenitors to their current localities. There is no context for studying any of these species on a regional scale to understand better their origins – they must be studied broadly.

Their conservation also calls for a unique strategy. Again, since they did not arise where they now grow and they arose repeatedly from the same parental taxa, the process should be the focus of conservation efforts. Rather than conserving taxa, in *Dactylorhiza* it seems more appropriate to preserve the habitats where hybridization has been occurring, but knowing that few hybrids are currently being formed in northern Europe means that more attention must be focused on appropriate sites in southern Europe where in general conservation efforts have not been as successful in the past.

LITERATURE CITED

Pillon, Y., M.F. Fay, M. Hedrén, D.S. Devey, A. Shipunov, M. van der Bank, R.M. Bateman & M.W. Chase. 2007. Insights into the evolution and biogeography of western European species complexes in *Dactylorhiza* (Orchidaceae). Taxon: in press.

Shipunov, A.B., M.F. Fay, Y. Pillon, R.M. Bateman & M.W. Chase. 2004. *Dactylorhiza* (Orchidaceae) in European Russia: combined molecular and morphological analysis. Amer. J. Bot. 91: 1419-1426.

Mark Chase received his undergraduate degree from Albion College, Michigan and his Ph.D. was from the University of Michigan (Ann Arbor) in 1985. His thesis was a monograph of *Leochilus* (Orchidaceae). He carried out post-doctoral research in molecular biology with Jeff Palmer at the University of Michigan. He then moved to the University of North Carolina and then after four year to the Royal Botanic Gardens, Kew, where he set up the program in molecular systematics. He became a member of the Royal Society in 2003 and Keeper (Director) of the Jodrell Laboratory in 2006.

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SEQUENCING RE-DEFINES *SPIRANTHES* RELATIONSHIPS, WITH IMPLICATIONS FOR RARE AND ENDANGERED TAXA

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Introduction

Species delimitation in the genus *Spiranthes* L.C. Richard (Spiranthinae, Cranichideae, Orchidoideae) has long been problematic, due mainly to morphological polymorphism confounded by hybridization and polyploidy, particularly in the *S. cernua* (L.) Rich. species complex (Correll 1950, Luer 1975, Sheviak 1982). Official records indicate that 462 taxa names have been used for *Spiranthes* historically (RBG-Kew 2006), but less than a tenth of those are recognized today, and there is still concern about the species status of some. Although *Spiranthes* is considered to have a worldwide distribution, only eight of these occur outside of temperate North America.

All 26 currently recognized *Spiranthes* taxa in the Flora of North America (Sheviak & Brown 2002) have some form of conservation listing in a U.S. state (except *S. casei* var. *novascotiae* Catling, found only in Canada), due mainly to tenuous occurrence at the edge of their range in those locations. The less-serious listing denominations include: Exploitatively Vulnerable, Rare, Sensitive, and Special Concern. Two unrecognized taxa are other exceptions – *S. amesiana* (either extirpated or a synonym for *S. torta* [Thunb.] Garay & H.R.Sweet) is Proposed Endangered by Florida due to endemicity and rarity, and newly described *S. sylvatica* P.M.Brown (Brown 2001a) has not been listed by any state.

Most of these *Spiranthes* taxa are also federally or state-listed as Threatened, Proposed Endangered, or Endangered (Table 1). However, some taxa, such as those endemic to or now limited to one or few locations, should be targeted for special protection. These include: *S. brevilabris* Lindl., *S. delitescens* Sheviak, *S. eatonii* Ames ex P.M.Br., *S. floridana* (Wherry) Cory, *S. infernalis* Sheviak, *S. parksii* Correll, and *S. torta*. Federally threatened *S. diluvialis* Sheviak is not endemic to one area, but rare throughout its range and unusual as an allopolyploid. Other taxa that can also be identified as genetically important as well as rare should be targeted for maintenance of biodiversity.

Molecular genetic techniques can provide a suite of markers from which to choose the scale of taxonomic discrimination required (Soltis & Soltis 1998, Soltis & Gitzendanner 1998, Avise 2004). Nucleotide sequencing, particularly of several genes in combination, is successfully used to address issues of phylogenetics and species delimitation, critical when conservation resources to protect threatened and endangered taxa must be focused. It is thus our goal in determining phylogenetic relationships among *Spiranthes* through sequence analysis to help identify these unique taxa and verify the taxonomic status of the endemic group members. Circumscribing the genetic individuality of these species of concern is a basic foundation on which to build further conservation efforts.

Methods

As part of a larger *Spiranthes* phylogeny project, all 27 taxa found in temperate North America have now been sampled except *S. casei* var. *novascotiae* and *S. ovalis* var. *ovalis*. Voucher specimens, when available, were deposited in the Clemson (SC) University herbarium (CLEMS). Three *Spiranthes* found in Europe and Asia (*S. aestivalis* [Poir.] Rich., *S. sinensis* [Pers.] Ames, *S. spiralis* [L.] Chevall.), as well as an outgroup taxon (*Sacoila lanceolata* var. *lanceolata* [Aubl.] Garay), were also included in the analyses.

DNA was extracted from the plant tissue, and four genes/regions representing all three genomes were sequenced according to protocols outlined in Dueck et al. (2005). The DNA segments analyzed include

TABLE 1. Critical conservation listings for *Spiranthes* in the U.S.

SPECIES	FEDERAL STATUS	GLOBAL RANKING	STATE STATUS	STATE RANKING	STATE	NOTES
S. brevilabris		**G1**	**E**	**S1**	FL	1 extant FL site
S. casei v. *casei*		**G4**	**E**	**S1**	NH, PA	
S. delitescens	**E**	**G1**	**HS**	**S1**	AZ	endemic to 1 AZ site
S. diluvialis	**T**	**G2**	**E** **T**	**S1** **S2, S1**	WA MT, NE	found in 8 states
S. eatonii			**PE**		FL	2 extant FL sites
S. floridana		**G1**	**PE**	**S1**	FL	1 extant FL site
S. infernalis		**G1**	**T**	**S1**	NV	endemic to 1 NV site
S. lacera			**T**		IA	
S. lacera v. *gracilis*		**G5**	**X/PE**		ME	
S. laciniata		**G4G5**	**E** **T/E** **T**	 **S1**	NJ NC FL SC	
S. longilabris		**G3**	**T** **T/PE**		NC FL	
S. lucida		**G5**	**E** **T/E** **T**	 **S1**	IL, IA, MD NC KY, ME, NH, TN	
S. magnicamporum		**G3G4**	**E** **T**	**S1**	GA, IN, NM KY	
S. ochroleuca		**G4G5**	**E** **T/E** **T**	 **S2**	MD, TN NC IN	
S. odorata		**G5**	**X/E** **E**		MD KY, TN	
S. ovalis v. *eros.*		**G5**	**E** **T**	 **S1**	FL, PA IA, MI, seUS	
S. ovalis v. *ovalis*		**G5**	**E**		FL	
S. parksii	**E**	**G3**	**E**	**S3**	TX	endemic to east TX
S. romanzoffiana		**G5**	**E** **E** **T**	**S1**	IN MA, IL, PA IA, OH	
S. torta		**G4**	**E**	**S1**	FL	endemic to south FL
S. tuberosa			**E** **T/E** **T**		RI NJ FL	
S. vernalis		**G5**	**E** **T**		IL, NH, NY, PA MA, IA	

Main source: http://plants.usda.gov/threat.html

X = Extirpated/Historical
E = Endangered
PE = Proposed Endangered
T = Threatened
HS = Highly Safeguarded

G1 = critically imperiled globally
G2 = imperiled globally due to rarity/vulnerability
G3 = very rare throughout range or found restricted locally
G4 = apparently secure globally but rare in some places
G5 = demonstrably secure globally but rare in some places
S1 = critically imperiled in state; **S2** = imperiled in state

two plastid regions – a non-coding section of *trnS-fM* and the *trn*L intron, one non-coding region in mitochondrial gene NAD7, and the nuclear ribosomal ITS region including ITS 1, 5.8S, and 2. Resulting electropherograms were contiged and edited, and the four matrices of consensus sequences were aligned. These data sets were then analyzed using the parsimony criterion in PAUP*, and support values for relationships were calculated by performing jackknife analyses of 5000 replicates. Since the separate gene trees were concordant, data were also combined and analyzed together, but only samples complete for all four genes were included.

Because the main project is still in progress, however, the four-gene cladogram shown here (from June 2006) does not include many samples subsequently collected. These later samples have been sequenced, but their analyses not rigorously tested yet, so their results will be discussed as preliminary findings only.

Results

Of the 123 total samples analyzed by June 2006, 99 samples were complete with all four gene sequences and thus were used to produce the figure included. Two taxa subsequently sampled (*S. porrifolia* Lindl., *S. torta*) were not included in this analysis, but their placement is discussed below in light of more recent analyses. Other taxa not included due to unavailability are *S. casei* var. *novascotiae*, *S. lacera* var. *lacera* (Raf.) Raf., and *S. ovalis* var. *ovalis* Lindl. Over 3500 base pairs (bp) were used in the combined analysis, and statistically the ITS 1-2 segment had the most variable (26%) and informative (18%) sites. However, the *trnS-fM* segment had the best-resolved topology for a single-gene tree (not shown).

A strict consensus cladogram of the *Spiranthes* phylogeny based on the four-gene combined analysis is shown in Figure 1, with jackknife support values added above branches. There is strong support (99%) for a division of the genus into two major groups with some strong species clades within each. The lower group, a paraphyletic grade of taxa, contains distinct species *S. infernalis* and *S. lucida* (H.H.Eaton) Ames, and a weakly supported *S. romanzoffiana* Cham. clade in which strongly supported *S. delitescens* and unsupported but separate *S. diluvialis* reside. In single trees from maternally inherited plastid and mitochondrial genes, *S. diluvialis* was within the same clade as S. *romanzoffiana*, but in the tree based on nuclear ITS sequences, it was within the clade containing *S. magnicamporum* Sheviak (not shown). A moderately supported division (81%) within the lower paraphyletic grade separates the latter five species from strong clades of combined *S. praecox* (Walter) S.Watson and *S. sylvatica* (no distinction between them shown from this analysis), all three Old World species, and *S. tuberosa* Raf. The upper group contains a moderately supported clade of closely related species, with *S. brevilabris* and *S. floridana* as distinct, but with *S. eatonii* and *S. lacera* as indistinguishable from each other. *S. vernalis* Engelm. & A.Gray, *S. laciniata* (Small) Ames, and *S. longilabris* Lindl. are monophyletic and thus strongly supported species. *S. ovalis* var. *erostellata* appears as a strongly supported subclade within a broad unsupported *S. magnicamporum-S. odorata* (Nutt.) Lindl. group. *S. odorata* is polyphyletic, appearing within no fewer than three different clades. The two remaining derived clades consist of moderately strong support (86%) for a *S. cernua-S. parksii* clade in which *S. parksii* is indistinguishable, and an unsupported clade with *S. casei* Catling & Cruise and *S. ochroleuca* (Rydb.) Rydb. separate but also unsupported. No distinct identity for *S. cernua* was supported from these samples and analysis.

Preliminary results from "work-in-progress" analyses (not shown) indicate some important findings for species of interest to conservation biologists. Differentiation of *S. eatonii* from *S. lacera* was not resolved by additional *S. lacera* samples. *S. porrifolia* is very closely related to *S. infernalis* and in fact may be hybridizing in the southwestern limits of its range, but the clade containing both species are distinct from and sister to *S. romanzoffiana*. Our samples of *S. torta* are sister to *S. laciniata* (and both to *S. brevilabris*) in the maternal genomes, but to *S. floridana* when the nuclear genome is included. And although inclusion of more samples of *S. cernua* is providing a better species identity for it along a northeastern Appalachian swath, *S. parksii* remains imbedded within the clade of southern *S. cernua*.

Discussion

For the first time, our phylogeny of *Spiranthes*, based on molecular data from all three genomes, is revealing the relationships among taxa found in the United States and abroad. Whereas several other aspects of the study are interesting, our goal here is to focus on known target species (endemic/endangered) and define any new species (unique/rare) of conservation concern.

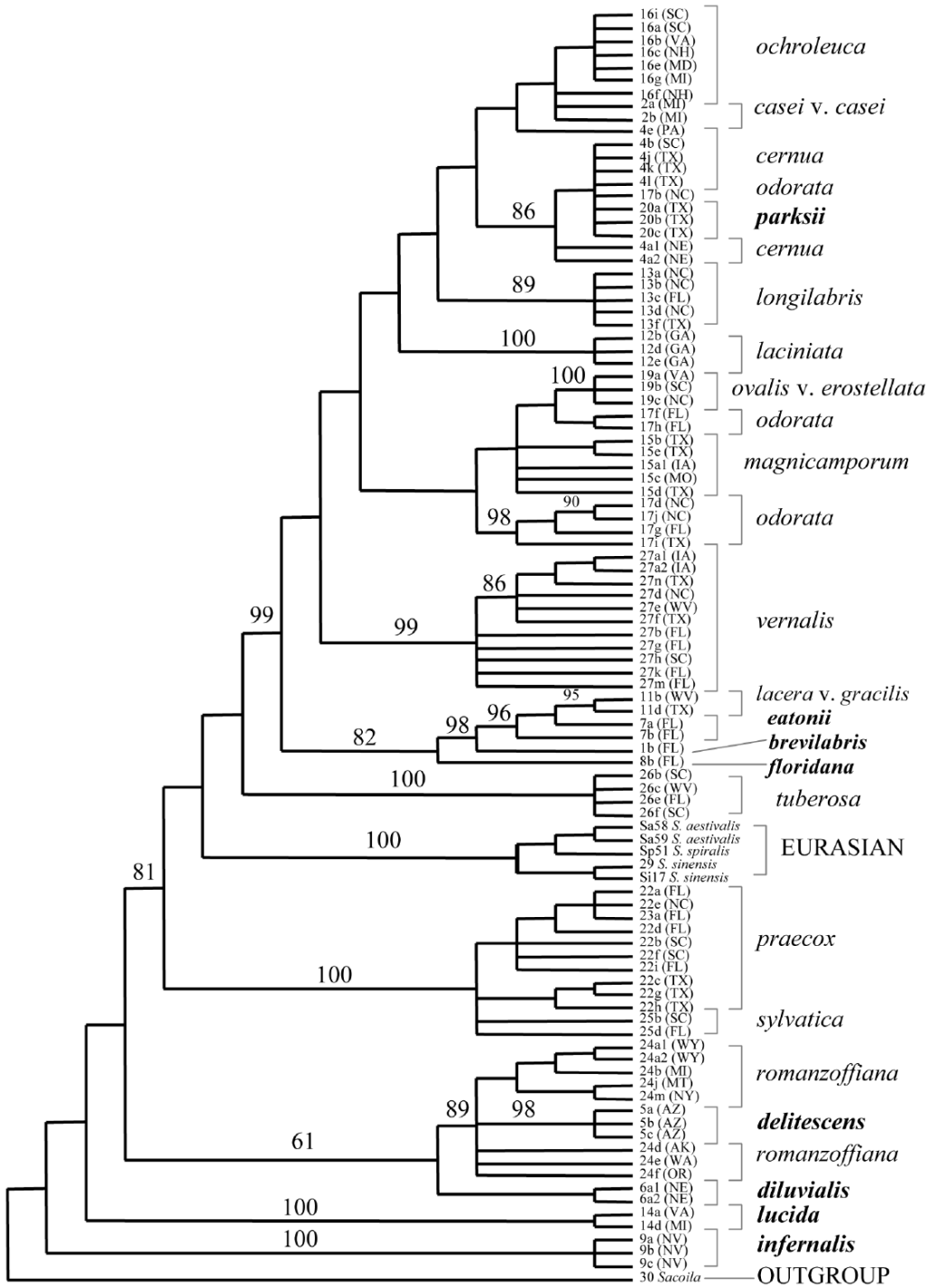

FIGURE 1. Strict consensus cladogram for taxa in *Spiranthes* genus, based on combined analysis of four genes/regions, from 489 trees (June 2006); jackknife support values >80 shown above branches; target species for conservation in **bold**. Two species subsequently sampled and analyzed but not included here are ***S. porrifolia*** and ***S. torta***, both also of conservation concern. Sample identification numbers and U.S. state of collection shown at branch tips.

Most importantly, federally listed *S. parksii*, endangered and endemic to east-central Texas, may simply be an aberrant form of the more widespread *S. cernua*, contrary to accepted taxonomy. Cladograms from more than 3500 characters show that individuals of southern *S. cernua* form a monophyletic clade that includes *S. parksii* within it. Thus, based on our data and the phylogenetic species concept, *S. parksii* may not warrant species status, and its protection may be questioned. The tetraploid chromosome number (2n=60) of *S. parksii* and inclusion firmly within the *S. cernua* complex (Sheviak & Brown 2002) suggests that polyploidy may play a role in its ambiguity.

In contrast, species status was supported by our data for the two other federally listed species, *S. diluvialis* (threatened) and especially *S. delitescens* (endangered), both members of a broad *S. romanzoffiana* clade. The previously documented allopolyploid origin of *S. diluvialis* was confirmed by these data, with *S. magnicamporum* verified as its paternal ancestor. Allopolyploidy has also been suggested for *S. delitescens* by Sheviak & Brown (2002) based on the same chromosome number configuration (2n=74), but affinity with other than *S. romanzoffiana* was not confirmed by our data.

The genetic identity of all other state-listed *Spiranthes* species in the endemic/endangered group except one was confirmed. Although Wherry separated *S. floridana* from *S. brevilabris* in 1931 (as *Ibidium floridanum*, later changed to *S. floridana* by Cory and emended by Brown [2001b]), the epithet *S. brevilabris* var. *floridana* has persisted. Our study shows these rare taxa to be distinct species, each worthy of separate recovery plans. However, from the few samples of *S. eatonii* sequenced, we were not able to distinguish it genetically from *S. lacera*. Extremely rare *S. torta* does appear to be a distinct species but closely related to the above four taxa, although there is some evidence for its hybridization with *S. laciniata*, which could be confirmed by further studies.

Three taxa have also been identified as genetically isolated by our study. Extremely rare *S. infernalis*, also a member of the endemic/endangered group, is a distinct subclade within a broader *S. porrifolia* complex near the ancestral root of the topology. While *S. porrifolia*'s range covers the West coast states, its occurrence is sporadic, so linkage with rarer *S. infernalis* enables more concern for its conservation, also. And *S. lucida*, listed by eight states, is perhaps the most unique taxon genetically as sister to all other *Spiranthes* in our recent preliminary work, and thus worthy of concerted conservation effort. We therefore suggest consideration of *S. infernalis* and *S. lucida* for federal listing status based on their basal position in the *Spiranthes* phylogeny, genetic uniqueness within the genus, and rarity.

These data demonstrate that molecular technologies have the power to elucidate genetic identity, focusing funding eligibility and conservation efforts such as *ex situ* propagation on the most appropriate subjects for maintenance of biodiversity.

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LITERATURE CITED

Avise, J.C. 2004. Molecular Markers, Natural History, and Evolution. Second Edition. Sinauer Associates, Sunderland, MA.

Brown, P.M. 2001a. Recent taxonomic and distributional notes from Florida 11: *Spiranthes sylvatica* P.M. Brown, a new species of ladies'-tresses from the southeastern United States. North Amer. Native Orch. J. **7** : 193-201.

Brown, P.M. 2001b. Recent taxonomic and distributional notes from Florida 9. North Amer. Native Orch. J. 7(1) : 91-98.

Correll, D.S. 1950. Native Orchids of North America North of Mexico. Chronica Botanica Co.

Dueck, L.A, J.A. Fowler, C.S. Hagen & T.C. Glenn. 2005. Genetic discrimination of *Spiranthes cernua* species complex in South Carolina. Selbyana 26 (1,2) : 145-154.

Luer, C.A. 1975. The Native Orchids of the United States and Canada excluding Florida. New York Botanical Garden, NY.

Sheviak, C.J. 1982. Biosystematic study of the *Spiranthes cernua* complex. Bulletin #448, New York State Museum. University of the State of New York, The State Education Department, Albany, NY.

Sheviak, C.J & P.M. Brown. 2002. Orchidaceae, Vol. 26, *Spiranthes*. Flora of North America. Published on the Internet at: http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=131021.

Soltis, P.S & M.A. Gitzendanner. 1998. Molecular systematics and the conservation of rare species. Conserv. Biol. 13 : 471-483.

Soltis, D.E. & P.S. Soltis. 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. *In* D.E. Soltis, P.S. Soltis & J.J. Doyle (eds.). Molecular Systematics of Plants II: DNA Sequencing. Kluwer Academic Publishers, Norwell, MA, pp. 1-42 .

Walters, C. 2005. Genetic relationships among *Spiranthes parksii* and congeneric species. M.Sc. thesis, Texas A&M University, College Station, Texas.

World Checklist of Selected Plant Families. 2006. The Board of Trustees of the Royal Botanic Gardens, Kew. Published on the Internet at: http://www.rbgkew.org.uk/wcsp/home.do, accessed 10/26/06.

Lucy Dueck, M.Sc., has been a Research Professional in molecular ecology at SREL, a field outpost for the University of Georgia, for over seven years. Her interest in *Spiranthes* phylogeny developed from producing a booklet on the wild orchids of South Carolina for outreach purposes. She obtained a grant from the AOS to pursue a thorough phylogenetic survey after completing a pilot study on selected *Spiranthes* in South Carolina.

Ken Cameron, Ph.D., is Director of the Lewis B. and Dorothy Cullman Program for Molecular Systematic Studies and an Associate Curator at The New York Botanical Garden. He has published extensively on the molecular systematics of various plant families, but maintains a primary research focus on orchids. In particular, he has applied DNA sequencing to questions of phylogeny for *Vanilla* and its relatives, as well as studies of Orchidaceae as a whole.

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MOLECULAR GENETIC DIAGNOSIS OF THE 'TAXONOMICALLY DIFFICULT' AUSTRALIAN ENDANGERED ORCHID, *MICROTIS ANGUSII*: AN EVALUATION OF THE UTILITY OF DNA BARCODING.

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KEY WORDS: Species diagnosis, barcoding, practical outcomes, clonality, Internal Transcribed Sequences (ITS), Single Nucleotide Polymorphisms (SNPs)

As species are the common currency for conservation efforts, their accurate description is essential for efficient preservation of biological diversity. The use of DNA Barcodes, short DNA sequences that evolve fast enough to differentiate species, has been proposed both for the discovery of new species and the identification of previously described species. The first objective remains controversial, with a strong argument that species discovery be based on 'Integrated Barcodes', including multiple sources of data (see Rubinoff 2006a). In contrast, once a species has been described, including for molecular sequence data at barcoding loci, the use of a DNA barcode may facilitate the identification of the species, particularly in cases where recognition based on easily-visualised characters is problematic.

Nonetheless, the use of the DNA Barcoding approach for species diagnosis pre-supposes a comprehensive understanding of the circumscription of the species under study. In reality this is very rarely true, as it requires the combination of multiple lines of investigation, which, in the case of orchids include pollinator observations and manual cross pollination, in addition to morphological and molecular character analysis (Peakall 2007). Thus, while ideally the sole use of DNA Barcoding should be limited to species diagnosis, in practice it may often yield further data to be set against the working hypothesis of species status, thus contributing to species delineation and discovery.

We present a study of the application of barcoding to the endangered Australian orchid, *Microtis angusii* (Flanagan *et al.* 2006). This species was recently described from a single location in New South Wales, Australia, consisting of approximately 100 plants (Jones 1996). *Microtis* species commonly exhibit clonal growth (Peakall & Beattie 1989, 1991), and it was highly likely that the plants present at the type location represented a small number of clones. Additionally, the site had been subject to various threatening processes such as road improvements and encroachment by invasive plants. *Microtis angusii* was listed as a nationally endangered species on Schedule 1 of the Australian Commonwealth *Endangered Species Protection Act 1992* in 1997.

The genus *Microtis* has been relatively neglected taxonomically, possibly because of their inconspicuous small, green, often ant-pollinated flowers. *Microtis angusii* is morphologically very similar to more common, widespread relatives, and easily confused, even by experienced field biologists. In accordance with the New South Wales Threatened Species Conservation Act, a recovery plan for the species was prepared in order to ensure self-sustaining populations in the wild. For this, the identification of further populations of *M. angusii* was highly desirable, but hindered by difficulties in species recognition.

Conservation practitioners identified six potential populations of *M. angusii*, and requested a genetic study to provide confirmation of their con-specific sta-

tus for the recovery plan. We investigated patterns of molecular genetic variation in both Amplified Fragment Length Polymorphisms (AFLPs) and DNA sequence (rDNA ITS) loci, compared to that of the type population and known examples of potentially confounding, congeneric species. The type population was invariable across 122 AFLP markers. Of the six potential populations only two were identified unambiguously as *M. angusii*, having identical ITS sequences and highly similar AFLP profiles. Three populations collected showed a high genetic affinity to the related species *M. parviflora*, including identical ITS sequences, while a fourth population was diagnosed, on the basis of the molecular data, to be *M. rara*.

A subset of samples from one of the populations was most similar to, but not identical to *M. angusii* across the genetic loci. This genetically-distinct clade may represent an additional previously-unknown species. Alternatively, given the clonal nature of the *Microtis* genus, this geographically distant population may represent a highly differentiated conspecific population. Clonality, in combination with high selfing rates due to restricted ant pollination (Peakall & Beattie 1989, 1991) are traits that will act to reduce effective population size, thereby enhancing the effects of genetic drift and so promoting higher levels of genetic differentiation between isolated populations than expected in a predominantly outbreeding species.

Whilst barcoding based on complete DNA sequence data is preferred in order to identify rare, differentiated haplotypes, extensive sequencing projects are expensive and beyond the financial capacity of many conservation programmes. In order to provide an economical alternative to full sequence characterization, we designed a rapid, PCR-based assay for the effective identification of *M. angusii* from single nucleotide polymorphism (SNP) differences seen in the study of sequence variation at the ITS locus (Flanagan *et al.* 2007). The assay was designed to be easily visualized on a standard agarose gel, avoiding the use of expensive restriction enzymes and DNA sequencing reagents and equipment.

An important aspect of this assay was its validation through the application of a 'blind trial'. Here the assay was applied to samples of disguised identity, including all ITS haplotypes identified in the original genetic survey, and samples from a previously uncharacterized population. *Microtis angusii* samples were successfully discriminated from amongst the several congeners, and the further, previously unknown, population was diagnosed as *M. angusii*. Sequencing of the ITS locus for these individuals confirmed this PCR diagnosis.

While these studies demonstrate the application of DNA Barcoding for species diagnosis of the endangered *M. angusii*, it must be emphasized that further morphological and ecological studies of the genus *Microtis* are sorely needed in order to unambiguously define the species boundaries in the genus. As has been recognized by Rubinoff (2006b), amongst other authors, sole reliance on DNA sequence data at one, or a few loci, may mislead conservation efforts, either by making decisions on species status based on characters that are not species-specific, or by diverting resources from broader studies that are ultimately more capable of providing robust species circumscriptions. It is imperative that, in the reality of limited funds for conservation research, priority must be given to studies that will have direct practical outcome in conservation management. A recent review suggested that genetic studies of clonal plants, plants with uncertain taxonomic status, and plants targeted for translocation were most likely to result in practical outcomes (Hogbin *et al.* 2000). Nonetheless, as the case of *Microtis angusii* shows, even in these scenarios a genetic study is not necessarily sufficient by itself.

LITERATURE CITED

Flanagan, N.S., R. Peakall, M.A. Clements & J.T. Otero. 2006. Conservation of taxonomically difficult species: the case of the Australian orchid, *Microtis angusii*. Conservation Genetics 7: 847-859.

Flanagan, N.S., R. Peakall, M.A. Clements & J.T. Otero. 2007. Identification of the endangered Australian orchid, *Microtis angusii* using an allele-specific PCR assay. Conservation Genetics. Online First.

Hogbin, P.M., R. Peakall & M.A. Sydes. 2000. Achieving practical outcomes from genetic studies of rare plants. Australian Journal of Botany 48: 375-382.

Jones, D.L. 1996. *Microtis angusii*, a new species of

Orchidaceae from Australia. The Orchadian 12: 10-12.
Peakall, R. 2007. Speciation in the Orchidaceae – confronting the challenges. Molecular Ecology. In press.
Peakall, R. & A.J. Beattie. 1989. Pollination of the orchid *Microtis parviflora* R.Br. by flightless worker ants. Functional Ecology 3: 515-522.
Peakall, R. & A.J Beattie. 1991. The genetic consequences of worker ant pollination on a self-compatible, clonal orchid. Evolution 45: 1837-1848.
Rubinoff, D. 2006a. DNA Barcoding Evolves into the Familiar. Conservation Biology 20: 1548-1549.
Rubinoff, D. 2006b. Utility of Mitochondrial DNA Barcodes in Species Conservation. Conservation Biology 20: 1026-1033.

Nicola Flanagan has a broad experience of evolutionary studies in the Orchidaceae, including species boundaries in the sexually-deceptive *Chiloglottis* orchids, orchid mycorrhizal specificity in the tropical *Ionopsis utricullariodes*, and patterns of genetic variation in *Vanilla* species.

Rod Peakall has interests spanning the fields of plant reproductive biology, population genetics, evolutionary biology and conservation biology and has worked on a range of plant and animals species. His current research is focused on the evolution of sexually deceptive orchids.

Mark Clements has extensively studied the taxonomy and evolutionary relationships of Australian native orchids.

Tupac Otero has interests in orchid biology and interactions, including reproductive biology, mycorrhizal interactions, evolutionary biology and conservation biology.

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MOLECULAR TOOLS AND DNA BARCODING FOR CONSERVATION

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KEY WORDS: Darwin Initiative, biodiversity, conservation

The Darwin Initiative (DI) for the Survival of Species promotes biodiversity conservation and sustainable use of resources around the world (http://www.darwin.gov.uk). The main goal of the DI is to assist countries rich in biodiversity but poor in resources with the conservation of biological diversity and implementation of the Biodiversity Convention. Projects funded under the DI are collaborative, involving either local institutions or communities in the host country in collaboration with a British institution. Here we present four Darwin Initiative projects using molecular tools for species identification, forensic use and conservation:

• the project entitled "Conservation and Monitoring of MesoAmerican orchids" (Ref. 14-001), is based on a partnership between the Lankester Botanica Garden in Costa Rica and the Royal Botanic Gardens Kew in UK.

• the project called "Molecular tools for promoting biodiversity in rainforest fragments of Borneo" (Ref. 10-025), is the result of a collaboration between the Institute of Tropical Biology and Conservation University Malaysia Sabah, the Forest Research Centre Sabah and the Yayasan Sabah and the University of York, the University of Leeds, the Natural History Museum in UK.

• for this project entitled "Certification to support conservation of endangered Mexican desert cacti"(Ref. 14-059), partners come from two universities in Mexico - Universidad Autonoma de Querétaro and Universidad Nacional Autónoma de México (UNAM) – and the University of Reading in UK.

• the project called "Building Genetic Forensic Capacity to reduce South Africa's illegal trade" (Ref. 13-018), involves researchers and students from the University of Kwazulu-Natal in South Africa and University of Sheffield in UK.

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FINDING A SUITABLE DNA BARCODE FOR MESOAMERICAN ORCHIDS

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KEY WORDS: DNA barcoding, orchids, Costa Rica, plastid genome, coding region, *trnH-psbA*

Introduction

Recently, DNA barcoding has emerged as an effective tool for species identification. This has the potential for many useful applications in conservation, such as biodiversity inventories, forensics and trade surveillance. It is being developed as an inexpensive and rapid molecular technique using short and standardized DNA sequences for species identification. The core idea of DNA barcoding is based on the fact that short pieces of DNA can be found that vary only to a minor degree within species, such that this variation is much less than between species (Savolainen *et al*. 2005). As proposed by Hebert *et al.* (2003), the DNA barcoding system for animals has been based upon sequence diversity in mitochondrial cytochrome *c* oxidase subunit 1 (COI or *cox1*). However, in land plants, the *cox1* gene has too low a rate of DNA sequence change to be used for species-level discrimination. The plastid genome of plants seems to be a better candidate for DNA barcoding, with enough variation to distinguish species and at the same time less intra- than inter-specific variability (Chase 2005, Cowan 2006). In 2005, Kress *et al.* proposed a non-coding plastid region, the *trnH-psbA* spacer, as a good barcode candidate. The Consortium for the Barcoding of Life (CBOL), via the Plant Working Group, has established another strategy to find a universal DNA barcode for land plants. A subset of six coding regions has been selected and is currently being tested in various plant taxa.

Our study is part of a project funded by the Darwin Initiative for the Survival of Species, which promotes biodiversity conservation and sustainable use of resources around the world (http://www.darwin.gov.uk). This project, based on a partnership between several academic and governmental authorities in Costa Rica with the Royal Botanic Gardens, Kew, in the UK, aims to record orchid diversity, establish long-term monitoring sites and undertake a pilot study on DNA barcoding for conservation and trade surveillance. Although some approaches to identify a DNA barcoding approach for land plants focused on a wide range of species around the world, e.g. the work lead by the Plant Working Group of CBOL, our work concentrates on a limited geographical area, Costa Rica, and a hyper-diverse family of plants, orchids. Costa Rica has one of the richest orchid floras in the world, with over 1300 species on a relatively small territory of 51,000 km^2. In spite of the fact that this country has a well-developed network of protected areas, with over 25% of its territory composed of protected forests and reserves, orchid floras remain under constant threat from factors such as deforestation and illegal trade. Furthermore, orchids are well known to be difficult to identify, particularly when they are sterile. Therefore, the use of a rapid and standardized DNA-based identification tool will be invaluable for many applications in conservation and to enforce international conventions such as the Biodiversity Convention (CBD) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Hence, among other activities of this project, we are currently working on the development of a DNA barcode for Mesoamerican orchids, in particular Costa Rican species.

Nuclear regions such as the internal transcribed spacer of the ribosomal DNA (ITS), although often highly variable in angiosperms, are not a practical option in several groups and show certain functional

limitation for DNA barcoding (Kress 2005). Both coding and non-coding plastid regions present various advantages (monomorphy, high copy number and highly diagnostic) and appear to be good candidates. One aim of barcoding is to find a "barcoding gap" between the intra- and inter- specific variation for the proposed regions (Meyer & Paulay 2005). We present here our preliminary results based on the comparison of different DNA plastid regions: the non-coding *trnH-psbA* spacer and five coding regions.

Materials and methods

We used standardized protocols for the PCR amplification and the sequencing available online on the RBG Kew website (http://www.kew.org/barcoding/protocols.html); all DNA samples came from the Kew DNA Bank (http://www.rbgkew.org.uk/data/dnaBank/homepage.html). The sampling covers 74 taxa representing 50 Mesoamerican orchid species and three temperate species as outgroups (from the North Temperate genus *Dactylorhiza*, Orchideae, Orchidoideae). We selected 47 species Costa Rican species and three species from other countries with a more northern distribution in Mesoamerica (from Mexico to Nicaragua).

To evaluate intra-specific variability, eleven of these species had multiple accessions (from two to seven). From the plastid genome, we sequenced the non-coding region *trnH-psbA* and portions of five DNA coding regions that have been put forward by the Plant Working Group of CBOL as potential universal barcodes for land plants, including *accD*, *rpoC1*, *rpoB*, *matK* and *ndhJ*. Altogether, these regions represent an aligned combined matrix of 3698 base pairs (bp) for 74 taxa.

We evaluated the inter- and intra-specific variation from a genetic distance matrix constructed using pair-wise Kimura 2 parameter (K2P) distances. The K2P model was used because it is simple and takes into account variable transition and transversion frequencies. Genetic distance between terminal taxa and their closest sister was used to characterize inter-specific divergence. The two most genetically distant individuals within each species were chosen to represent intra-specific divergence. We compared phylogenetic trees constructed using neighbour joining and parsimony methods. We also combined gene regions to evaluate the potential of a multi-locus barcode.

Results and discussion

Amplification was generally successful with all the regions tested. The only region that presented significant difficulties was *trnH-psbA;* there were alignment problems due to high levels of length variation. The sequence variability within and between species for all gene regions appears to overlap considerably, and, thus, these data do not show any evidence that there is a barcoding gap. Species groupings within neighbour joining and parsimony trees showed no topological differences. At the intra-specific level, the three gene regions that provided the greatest resolution were *matK*, *trnH-psbA* and *rpoB*, grouping over 50% of the eleven species with replicates into monophyletic groups. Among all combinations of regions tested as multi-locus barcodes, a "triplet" of *rpoC1*, *rpoB* and *matK* appeared to provide the best result and grouped all accessions of individuals correctly (Table 1).

Conclusion

As has been found in many plant groups (palms etc.), orchids exhibit low inter-specific sequence divergence, and there is no "barcode gap" between intra- and inter-specific data. However, results from the regions evaluated here show it is possible to

TABLE 1. Number of intra-specific species groupings per gene region from a neighbour joining tree (based on 11 species with replicates).

All regions are coding except for *trnH-psbA*.

Gene regions	Number of species groupings
accD	3 (27.3%)
matK	10 (90.9%)
ndhJ	1 (9.1%)
rpoB	6 (54.5%)
rpoC1	4 (36.4%)
trnH-psbA	8 (72.7%)
Triplet 1 (*rpoC1+rpoB+matK*)	11 (100%)
Triplet 2 (*rpoC1+matK+trnH-psbA*)	10 (90.9%)
Triplet 3 (*rpoB+matK+trnH-psbA*)	10 (90.9%)

group species replicates together, which is a basic requirement for a barcode identification tool. From the NJ reconstruction, the three best regions presenting the highest sequence variation and the best resolution at the species level are *rpoB*, *trnH-psbA* and *matK*.

It is clear that no single region will be sufficient as an efficient and universal barcode for orchids. A multi-locus barcode, based on two or three plastid regions, seems to be the most realistic and effective solution for the identification of Mesoamerican orchids. Our results show that a "triplet" of regions would be successful with a combination of regions like *rpoB*, *matK*, *trnH-psbA* or *rpoC1*. The next step for a multi-barcode will depend on the choice of using only coding regions or including a non-coding gene like *trnH-psbA*, although this gene presents practical complications with alignment.

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LITERATURE CITED

Chase, M.W., N. Salamin, M. Wilkinson, J. M. Dunwell, R.P. Kesanakurthi, N. Haidar & V. Savolainen. 2005. Land plants and DNA barcodes: short-term and long-term goals. Phil. Trans. R. Soc. B 360: 1889–1895.

Cowan, R.S., M.W.Chase, J.W.Kress & V. Savolainen. 2006. 300,000 species to identify: problems, progress, and prospects in DNA barcoding of land plants. Taxon 55: 611–616.

Hebert, P.D.N., S. Ratnasingham & J.R. De Waard. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. B 270: S96–S99.

Kress, J.W., K.J.Wurdack, E. A. Zimmer, L. A.Weigt & D. H. Janzen. 2005 Use of DNA barcodes to identify flowering plants. Proc. Natl Acad. Sci. USA 102: 8369–8374.

Meyer, C.P. & G. Paulay. 2005. DNA barcoding: Error rates based on comprehensive sampling. PLoS Biol 3: 2229-2238.

Savolainen, V., R.S.Cowan, A.P. Vogler, G.K.Roderick & R. Lane. 2005 Towards writing the encyclopaedia of life: an introduction to DNA barcoding. Phil. Trans. R. Soc. B 360: 1805–1811.

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RE-EVALUATION OF LIFESPAN IN A NEOTROPICAL ORCHID: AN ELEVEN YEARS SURVEY

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KEY WORDS: epiphyte; Kaplan-Meier; *Lepanthes*; Puerto Rico

Lifespan as a variable of the total survivorship period of individuals is infrequently studied. When studied it is often limited to investigation of the effect of some environmental variable on the lifespan of short lived morphological unit of the plant such as leaves or flowers. However, it is of crucial importance for determining the likelihood of survival of populations (Calder 1983, Tremblay, 2000) and should be part of any population viability analysis (PVA). Moreover evaluation of the mean (or medium) life and variance (or quartile) in lifespan is a variable of vital importance when considered for analyses of the likelihood of natural selection and genetic drift (Barrowclough & Rockwell 1993, Merila & Sheldon 2000, Crognier 2003). Many parameters are correlated with lifespan such as effective population size and lifetime reproductive success. Lifespan has a positive correlation with reproductive success, which is a consequence that individuals with longer lifespan have a higher probability of having offspring. Moreover, the larger the variance in lifespan the larger the variance in reproductive success and consequently effects the effective population size (Barrowclough & Rockwell, 1993; Tremblay & Ackerman, 2001).

Tremblay (2000) published a three years survey of lifespan in four species of *Lepanthes* including *L. caritensis*, however, after the three years survey most juveniles and adults were still alive (64 and 91%, respectively), while most seedlings died during the survey period with only 25% still alive at the end of the survey. As a result, the survivorship curve only represented a partial life history of the species with a maximum possible lifespan as function of the survey period. Limited survey's can easily bias the results, which can suggest smaller mean and variance in the projected lifespan. A second preoccupation with the previously published paper is that the number of seedling surveyed was very small and consequently the estimates could have been affected from random mortality and survivorship and not be a good representation of the typical lifespan of this life stage. Thus in this survey we present a continuation of the survey of one of the species, *Lepanthes caritensis* Tremblay & Ackerman and include a third population to the previously two studied with a total survey period of approximately eleven years for the first two populations.

Specifically we determined if mean and variance in lifespan from the surveyed populations and life stages are similar to the first 3 years survey. Secondly we evaluated if the survivorship among populations is equal. Thirdly, we investigated if the life stage and size (number of leaves) are determining factor in survivorship.

Methods

STUDY SYSTEM. *Lepanthes caritensis* is an endemic epiphytic, caespitose, and host specific orchid of 0.8 to 4.4 cm tall and its stems are appressed to the phorophyte. For description of the species and basic ecology refer to Tremblay & Ackerman 1993, Tremblay 1997a, 1997b, and Tremblay *et al.,* 1998.

STUDY AREA. This species occurs in a Subtropical Wet Forest of the Holdridge Life Zone System (Ewel & Wetmore 1973) and is found in the municipality of Patillas, Puerto Rico in the Carite Forest (Tremblay & Ackerman 1993). The plants are found at approximately 615m asl (maximum of Puerto Rico is 1100

TABLE 1. Sample size and sampled period for *L. caritensis* at three sites in the Carite Forest in Puerto Rico. Diameter at breast height (DBH) of the phorophyte (cm), and approximate sky visibility using a densiometer (% open sky).

Population	Number of individuals	Month survey started	Month survey ended	DBH of tree	Sky Visibility
1	184	September-94	August-06	43	36.40
2	111	September-94	August-06	45	32.24
3	222	May-02	August-06	67	26.00

asl) in an area that was apparently never logged. Annual mean temperature and rainfall are between 18 and 24˚C, and 2200 mm respectively (Ewel & Wetmore 1973). Rain is more or less consistent throughout the year with a slight increase in the winter period.

DATA COLLECTION. We censored a total of 517 individuals on an irregular basis for over 11 years of which approximately six years were surveyed monthly (Table 1). We numbered all individuals with plastic tags as described in Tremblay 2000. At each survey period the number of leaves of each individual was counted and the stage of the individual was noted. Individuals from all populations where identified as seedlings, juveniles, and adults, where seedlings are small plants without lepanthiform sheets on the petioles of any of the leaves, juveniles are individuals with at least one lepanthiform sheath on the petiole without evidence of past or current reproduction, and adults encompassed individuals that are currently reproductive (active inflorescences) or have been in the past (inflorescences may persist for years after they have been active). The identification of plant stages follow previous studies (Tremblay 2000; Tremblay & Hutchings 2003; and Rivera Gomez *et al.,* 2006). The numbers of individuals alive at the end of the survey varied from 39 to 113.

The individuals from the three populations are all found on older individuals of the tree *Micropholis guyanensis* (A. DC.) Pierre 1891 (Sapotaceae). Orchids are only found on the largest individuals of this species (Tremblay *et al*., 1998) and the three populations surveyed were on trees with DBH above 43 cm (Table 1).

INDICES MEASURED AND SOFTWARE USED. Comparison of the survivorship probability for all population and stages where evaluated by using proportional hazard or Cox's model, methods that lets you define models having failure time as the response variable with right-censoring and time-independent covariates. All tests including for multiple samples and the Kaplan-Meier's equation estimates were analyzed using the statistical softwares Statistica and JMP (Kaplan and Meier, 1958; Statsoft, 1994; survival analysis model; SAS Institute Inc., 2004, JMP v.5.1.2).

Results

GENERAL PATTERN OF SURVIVORSHIP. Combining data from all three populations and stages the mean death rate per year in this specie is 5.1% per year. The mean lifespan (se) was estimated at 1388 $\pm$ 74.2 days (approx 3.8 yrs), and median lifespan at 846 days (approx. 2.3 yrs; Table 2, Fig.1).

DIFFERENCES IN LIFESPAN AMONG POPULATIONS. Mean death rate per year by populations ranged from 4.1% to 18.0% per year. The two populations that were surveyed for 11 years had similar death rates while the third population had a higher death rate (Table 1, 2). The mean (s.e.) expected lifespan by population ranged from 739 $\pm$ 39 to 1544 $\pm$ 119 days (Table 3). We found a significant difference in the lifespan between all populations (Log-rank Test, $\chi^2 = 9.2846$, df = 2, p = 0.0096). Population two had the longest estimated lifespan while population three had the shortest estimated lifespan (Fig.2).

DIFFERENCES IN LIFESPAN AMONG LIFE STAGES. Summing the individuals from the different stages of the three populations we found that the shortest mean lifespan (se) was observed in seedlings (286 $\pm$ 35.7) and the longest for adults (2037.1 $\pm$ 111.3; Table 2, Fig.3). The lifespan of the different stages were significantly different (Log –rank test, $\chi^2 = 260.2715$, df = 2, $p < 0.0001$). Seedlings are more at risk from failing than juveniles or adults.

TABLE 2. Total numbers of days surveyed, cumulative proportion surviving at the end of the survey, number of all individuals alive at the end of the survey, median, mean, and the standard error of the mean lifespan for all population and stages. (* = bias mean estimates as a result of censored observation (individuals still alive) in the final survey time, for this reason estimates are a lower bound for the true mean).

Population	Number of days surveyed	Cumulative proportion surviving (%)	Number of individuals of the mean	Median survivorship alive at the end of the survey	Mean	Standard error
1	4346	10.2	53	728	1544*	119
2	4346	7.4	39	1246	1244*	114
3	1567	26.7	113	653	739*	39
Combined		9.04	205	846	1388*	74
Stage						
Seedling		7.9	32	154	287	36
Juvenile		1.2	79	688	783	71
Adult		17.2	94	1246	2037*	111

THE EFFECT OF NUMBER OF LEAVES ON THE SURVIVORSHIP OF INDIVIDUALS. The number of leaves affected the survivorship within stage categories. The mean lifespan is dependent on the number of leaves in juveniles ranging from 539 to 1080 days and for adults we found that the mean lifespan (se) varies from 1653 to 2196 (Table 4). The median lifespan varied from 485 to 688 days for juveniles and 1120 to 1246 for adults, noting that the median for individuals with five or more leaves do not have an estimated value because more than 50% of individuals were still alive at the end of the 11 years survey.

Discussion

We found that in general that the mean lifespan of *Lepanthes caritensis* is approximately 3.8 years, which denotes that most of the individuals we've been studying had die in our 11 years survey. After the 3 yrs survey the lifespan for *L. caritensis* had been estimated at 1245 days (3.4 yrs; Tremblay 2000), which is clearly very close to the eleven yrs survey. The main effect on the length of the lifespan comes from the short lifespan of seedlings that influences the length of the species lifespan. The life history curve is a typical type two curve where the rate of change is fairly constant. Type one curve is for organisms living out their full physiological lifetime, for example *Rhododendron maximum*, and type three curve is for organisms experiencing high mortality and low survivorship in early stages like *Spergula vernalis* (Smith 1990).

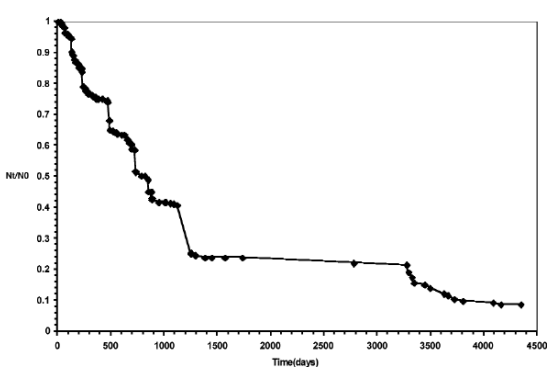

FIGURE 1. Survival curve of *Lepanthes caritensis* from an 11 yrs survey from 571 number of individuals. All populations and stages combined. Nt/No = the number of individuals at time t over the number of individual at the beginning of the survey.

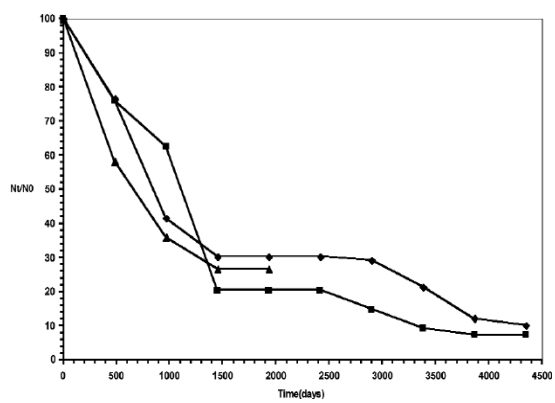

FIGURE 2. Cumulative proportion surviving for all populations of *L. caritensis*. Population 3 survey period was shorter. Nt/No = the number of individuals at time t over the number of individual at the beginning of the survey. Diamonds = pop. 1, squares = pop. 2, triangles = pop. 3.

TABLE 3. Percent death and mean lifespan (year) of *Lepanthes caritensis* and the sample size for estimating the parameter.

Population	Percent death/year	Mean lifespan (yrs)	Sample Size
1	7.5	4.2	184
2	7.9	3.4	111
3	18.0	2.0	222
Combined	7.7	3.8	517
Stages			
Seedling	45.3	0.8	96
Juvenile	9.5	2.1	197
Adult	7.0	5.6	224

TABLE 4. The lifespan of individuals with varying number of leaves and life stages, mean lifespan (se) and median time for individuals by stage (J = Juveniles, A = Adults). (* = Bias estimate as some individuals still alive at the end of the survey).

Number of leaves	Stage (N)	Sample size	Mean	Standard Error	Median Time
1	J	38	539	62	688
2	J	69	718	118	530
3	J	65	877	125	728
4	J	18	988	303	689
≥ 5	J	7	1080	502	485
1	A	34	1653*	285	1120
2	A	68	1978*	189	1246
3	A	63	1841*	178	1246
4	A	30	2023*	277	1246
≥ 5	A	20	2196*	265	--

The behavioral pattern of survivorship between the three populations demonstrates that the two longest surveyed populations behave similarly while the newly added site had a shorter lifespan. In the previous study the median survivorship (1245 days) in the second population was the same as in this analysis (1246 days), while the median lifespan in population one was much lower (1245 days as compared to 728 days after the 11 yrs survey). The newly added population has a lower median lifespan as compared to population one (653 days). The reduce survivorship in this population could be influenced by the light availability; the proportion of visible sky in the third population is reduced, consequently higher shaded area (Table 2).

The life stages (seedling, juvenile or adult) are informative in determining the plant's lifespan. Seedlings have short lifespan as compared to juveniles (2.7 times longer lifespan than seedlings), while adults also differ from juveniles (2.6 times longer lifespan). In the previous survey estimates of lifespan were not available because too few individuals had perished during the survey period (Tremblay 2000). The increase survey period allowed for gathering estimates on the mean and median in lifespan of the different stages of this species.

The numbers of leaves in *Lepanthes caritensis* makes a significant difference in the plants lifespan. There is a correlation between lifespan and the number of leaves held by an individual. The number of leaves is related to the total leaf area and consequently these individuals are more likely to survive environmentally stressful period or partial herbivory (rare in this species).

The addition of 8 more years to the survey for estimating *Lepanthes caritensis* survivorship had little effect on the estimators of seedlings, however the addi-

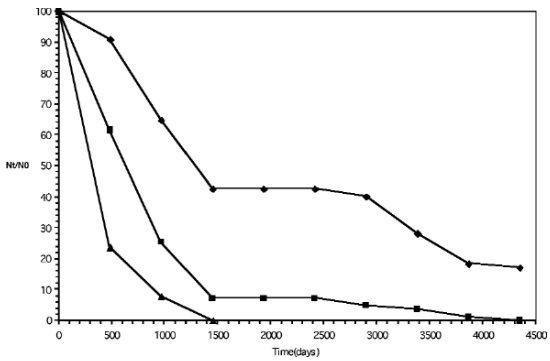

FIGURE 3- Cumulative proportion surviving for all three stages of *L. caritensis*. Nt/No = the number of individuals at time t over the number of individual at the beginning of the survey. Diamonds = Seedlings, squares = Juveniles, triangles = Adults.

tional survey suggest that juveniles and adults have a longer lifespan than predicted from the three year survey. Moreover, the number of leaves has a large impact of survivorship probabilities and thus could also be used a surrogate method for predicting lifespan instead of stages. Assuming constant mortality of adults at 7% mortality *per annum* and a Weibull survivorship distribution it is expected that 10% of adults survive 12.2 yrs and 1% will survive 21.5 yrs. Consequently a few individuals will likely have a very long lifespan as compared to most individuals and likely leave an excess of progeny as compared to most adults, resulting in a high variance in reproductive success.

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LITERATURE CITED

Barrowclough, G.F. & R.F. Rockwell 1993. Variance in Lifetime reproductive success: estimation based on demographic data. Amer. Nat. 141: 281-295.

Calder, W.A. 1983. Reproductive Success and Average Lifetime Natality. Oikos, 41: 288-289.

Crognier, E. 2003. Reproductive success: Which meaning? Am. J. Hum. Biol. 15: 253-260.

Ewell, J. & J. Whitmore 1973. The ecological life zones of Puerto Rico and the U.S. Virgin Islands. Inst. Tropical Forestry of Puerto Rico Res. Paper ITF-18. 72 pp.

Kaplan, E.L. & P. Meier 1958. Nonparametric estimation from incomplete observations." J. Am. Stat. Assoc. 53: 457-481.

Merila, J. & B.C. Sheldon 2000. Lifetime reproductive success and heritability in nature. Am. Nat. 155: 301-310.

Rivera Gómez, N., R.L. Tremblay & E. Meléndez-Ackerman. 2006. Density dependent effects in a lithophytic and epiphytic orchid. Folia GeoBotanica 41: 107-120.

Smith, R.L. 1990. Ecology and field biology. HarperCollins Publishers Inc. 4th edition. 341-347.

Tremblay, R.L. 1997a. Morphological variance among populations of three tropical orchids with restricted gene flow. Plant Species Biol. 12:85-96.

Tremblay, R.L. 1997b. *Lepanthes caritensis*, an endangered orchid: no sex, no future? Selbyana 18: 160-166.

Tremblay, R.L. 2000. Plant longevity in four species of *Lepanthes* (Pleurothallidinae: Orchidaceae). Lindleyana 15: 257-266.

Tremblay, R.L. & J. D. Ackerman. 1993. A new species of *Lepanthes* from Puerto Rico. Brittonia 45: 339-342.

Tremblay, R. L. & J. D. Ackerman. 2001. Gene flow and effective population size in *Lepanthes* (Orchidaceae): A Case for Genetic Drift. Biol. J. Linnean Soc. 72: 47 - 62.

Tremblay, R.L. & M.J. Hutchings. 2003. Population dynamics in orchid conservation: a review of analytical methods based on the rare species *Lepanthes eltoroensis*. Pp. 183-204 *in*: K. Dixon, S. Kell, R. Barrett & P. Cribb (eds.). Orchid Conservation. Natural History Publications (Borneo). Kota Kinabalu, Sabah, Malaysia. .

Tremblay, R.L., J. Zimmerman, L. Lebrón, P. Bayman, I. Sastre, F. Axelrod & J. Alers-García. 1998. Host specificity and low reproductive success in the rare endemic Puerto Rican orchid *Lepanthes caritensis* (Orchidaceae). Biol. Cons. 85:297-304.

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THE SPECIES-AREA-ENERGY RELATIONSHIP IN ORCHIDS

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ABSTRACT. Area, energy available and latitude are the main factors influencing species richness: (1) species richness increases with area – the species-area relationship (SAR); (2) according to the species-energy relationship (SER) the energy available to an assemblage (i.e. that which it can turn into biomass) at a particular spatial resolution influences the species richness; (3) there are more species per unit area in the tropics than in the temperate regions. To test the relative importance of area, energy available and latitude on species richness, we have collected data on species richness of orchids for various areas in the world and calculated the mean Normalized Difference Vegetation Index (NDVI) as a measure of energy availability in these areas. We show that area considered is always very important, and that latitude is more important than energy available.

KEY WORDS: orchids, species-energy relationship, NDVI

Introduction

Species–energy theory predicts a positive relationship between species richness and available energy (Brown 1981, Wright 1983, Wright *et al*. 1993). Species richness of a variety of taxa has been shown to increase with various amounts of available energy including net primary productivity (Hutchinson 1959, Brown 1981, Wright 1983, Guegan *et al*. 1998, Kaspari *et al.* 2000), potential and actual evapotranspiration (Rosenzweig 1968, Lieth 1975, Wright 1983, Currie & Paquin 1987, Currie 1991, Francis & Currie 2003) and precipitation (Brown & Davidson 1977). According to the *area hypothesis* (Connor & McCoy 1979, Wright 1983) larger areas contain more resources, which may support larger populations of each species, resulting in lower extinction rates and ultimately in more species. Similarly, the *more individuals hypothesis* (Wright 1983, Srivastava & Lawton 1998, Gaston 2000, Kaspari *et al.* 2003) assumes that there is a direct relationship between energy availability, the overall amount of resources in an area, the total number of individuals that can be maintained, and consequently the number of species. The *energy limitation theory* maintains that primary productivity is higher, because the tropics usually receive higher solar radiation and precipitation. This provides a wider resource base and enables more species to co-occur by increasing population sizes (Connell & Orias 1964, Wright 1983).

To test the relative importance of area, energy available and latitude on species richness, we have collected data on species richness of orchids for various areas in the world and calculated the mean Normalized Difference Vegetation Index (NDVI) as a measure of energy availability in these areas. We show that area considered is always very important, and that latitude is more important than energy available.

Methods

The numbers of orchid species recorded from 116 locations (countries or parts thereof) were obtained from a literature search. The areas of these locations were obtained from The Columbia Gazetteer of the World (Cohen 1998). Mean latitude of each location was calculated as the centroid of the area considered. We considered four regions: Africa, Eurasia, America and whole world.

The Normalized Difference Vegetation Index (NDVI) was used as a measure of energy available to

TABLE 1. Relationship between species richness of orchids from the regions and area and mean NDVI.

Region	Ln area	Ln mean NDVI	R^2	p-level
Africa	$F_{1,10}$ 2.9	$F_{1,10}$ 20.7**	0.676	0.0036
Eurasia	$F_{1,58}$ 0.2	$F_{1,58}$ 9.6**	0.155	0.0077
Whole America	$F_{1,39}$ 3.1	$F_{1,39}$ 0.6	0.108	0.1085
Whole world	$F_{1,113}$ 5.4*	$F_{1,113}$ 0.3	0.053	0.0461

*P < 0.5; **P < 0.01; ***P < 0.001; ****P < 0.0001

TABLE 2. Relationship between species richness of orchids from the regions and area.

Region	Ln area	Ln max NDVI	R^2	p-level
Africa	$F_{1,10}$ 2.6	$F_{1,10}$ 23.6***	0.704	0.0023
Eurasia	$F_{1,58}$ 0.2	$F_{1,58}$ 9.7**	0.155	0.0076
Whole America	$F_{1,39}$ 3.9	$F_{1,39}$ 0.0	0.094	0.1450
Whole world	$F_{1,113}$ 5.7*	$F_{1,113}$ 0.1	0.052	0.0491

*P < 0.5; **P < 0.01; ***P < 0.001; ****P < 0.0001

TABLE 3. Relationship between species richness of orchids from the regions and area and latitude.

Region	Ln area	Latitude	R^2	p-level
Africa	$F_{1,10}$ 0.9	$F_{1,10}$ 9.5*	0.490	0.0344
Eurasia	$F_{1,58}$ 3.8	$F_{1,58}$ 99.5****	0.637	0.0000
Whole America	$F_{1,39}$ 0.9	$F_{1,39}$ 8.5**	0.256	0.0031
Whole world	$F_{1,113}$ 0.5	$F_{1,113}$ 47.2****	0.331	0.0000

*P < 0.5; **P < 0.01; ***P < 0.001; ****P < 0.0001

an assemblage. NDVI is strongly positively correlated with green-leaf biomass, green-leaf area, and absorbed photosynthetically active radiation. This index has been viewed as providing reasonable representations of net primary productivity and vegetative growth of terrestrial ecosystems at the continental and global scale (Ustin *et al.* 1991, Kerr & Ostrovsky 2003), and thus as a suitable measure of the energy available to consumers. NDVI is derived from the visible and near infrared channel reflectances (0.58 to 0.68 μm and 0.73 to 1.10 μm, respectively). It is a dimensionless number with typical range from –0.200 to 0.730. This data set is produced as part of the NOAA/NASA Pathfinder AVHRR Land program (see http://disc.gsfc.nasa.gov/interdisc/readmes/pal_NDVI.shtml) and month data sets are available from the years 1981-1994. We used mean and maximum NDVI values from the vegetation season in 1994 (mean January – April NDVI for the southern hemisphere and May – August NDVI for the northern hemisphere) for the analyses.

The number of species, area and mean or maximum NDVI for each location were log transformed. We used the Statistica software (vs. 5.5, StatSoft, Inc., Tulsa, USA) for plotting 3D Surface Linear Plots with X-axis: ln(area); Y-axis: ln(mean NDVI), ln(max NDVI) or latitude; Z-axis: ln(number of species).

To determine the influence of area, NDVI or latitude on species richness we used Multiple Regression in General Linear Models (Statistica vs. 5.5, StatSoft, Inc., Tulsa, USA) with the number of species (ln(species richness)) as dependent variable and ln(area) and ln(mean NDVI), ln(max NDVI) or latitude as predictors.

For each region, linear regression was then fitted to the dependence of the logged number of species in location i, ln(species richness_i), and

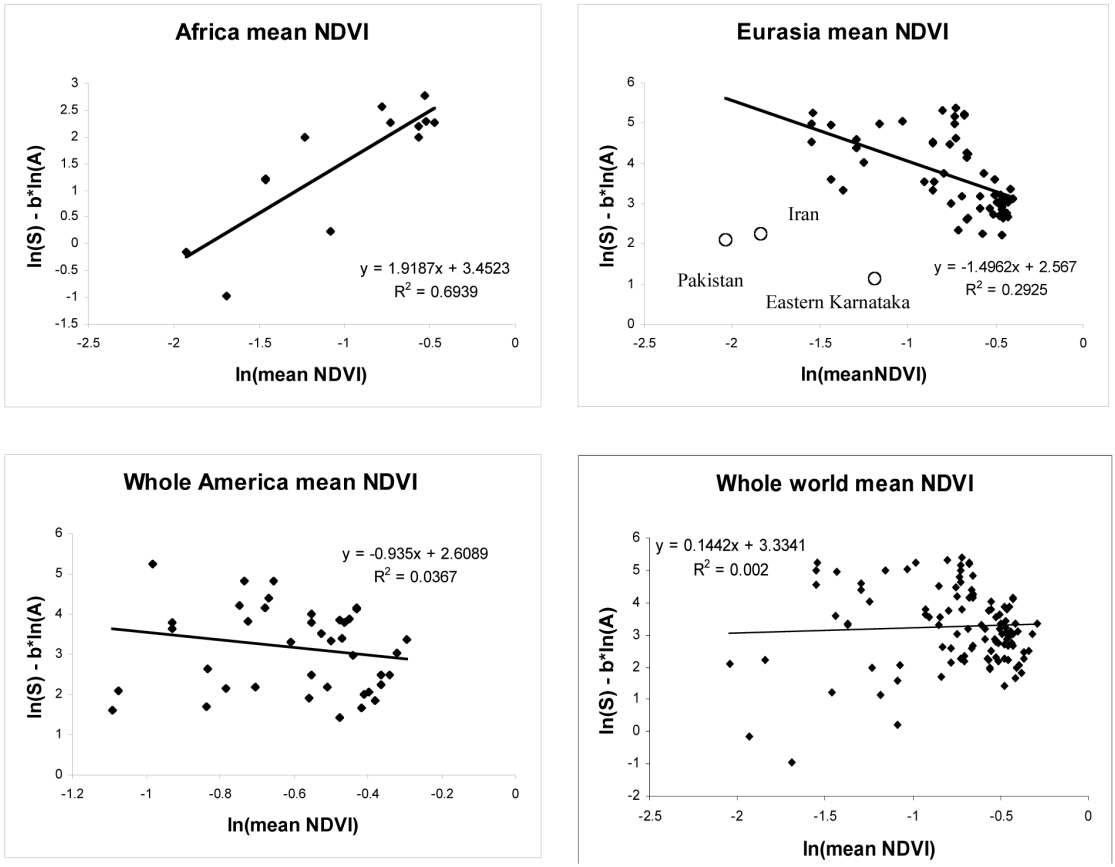

FIGURE 1. Relationship between logarithmically transformed mean NDVI and species richness per unit area.

logged area in location i, ln(area$_i$): ln(species richness$_i$) =a+b*ln(area$_i$). The b-values so obtained were then used to eliminate the influence of area on the number of orchid species at each location: we used the estimated number of species per unit area, ln(species richness$_i$)-b*ln(area$_i$), in each location instead of ln(species richness$_i$) for further analyses.

Results

Multiple regression in GLM with ln(species richness) as dependent factor and ln(area) and ln(mean NDVI) or ln(max NDVI) as predictors has shown a significant influence of ln(mean NDVI) and ln(max NDVI) only in Africa and in Eurasia (Tables 1, 2). Ln(area) significantly affected species richness only in the data set from the whole world (Tables 1, 2).

When the logged number of species per unit area was considered, a positive influence of ln(mean NDVI) or ln(max NDVI) was recorded only in Africa (Figures 1 and 2). A negative influence of ln(mean NDVI) and ln(max NDVI) on the species richness was recorded in Eurasia, where species richness decreases with NDVI (Figures 1, 2). Data sets from America and whole world did not show any significant trend (Figures 1, 2).

A significant influence of latitude was recorded in all regions (Table 3). From Figure 3 it is obvious that species richness decreases with latitude. Somalia and Sudan from Africa, Eastern Karnataka from Eurasia and Somalia, Sudan, Eastern Karnataka, Ethiopia and Morocco from the whole world data set were excluded as outliers in these figures. The reason for the exclusion will be discussed in the Discussion. No difference was found between temperate South and North America. Absolute value of latitude was used

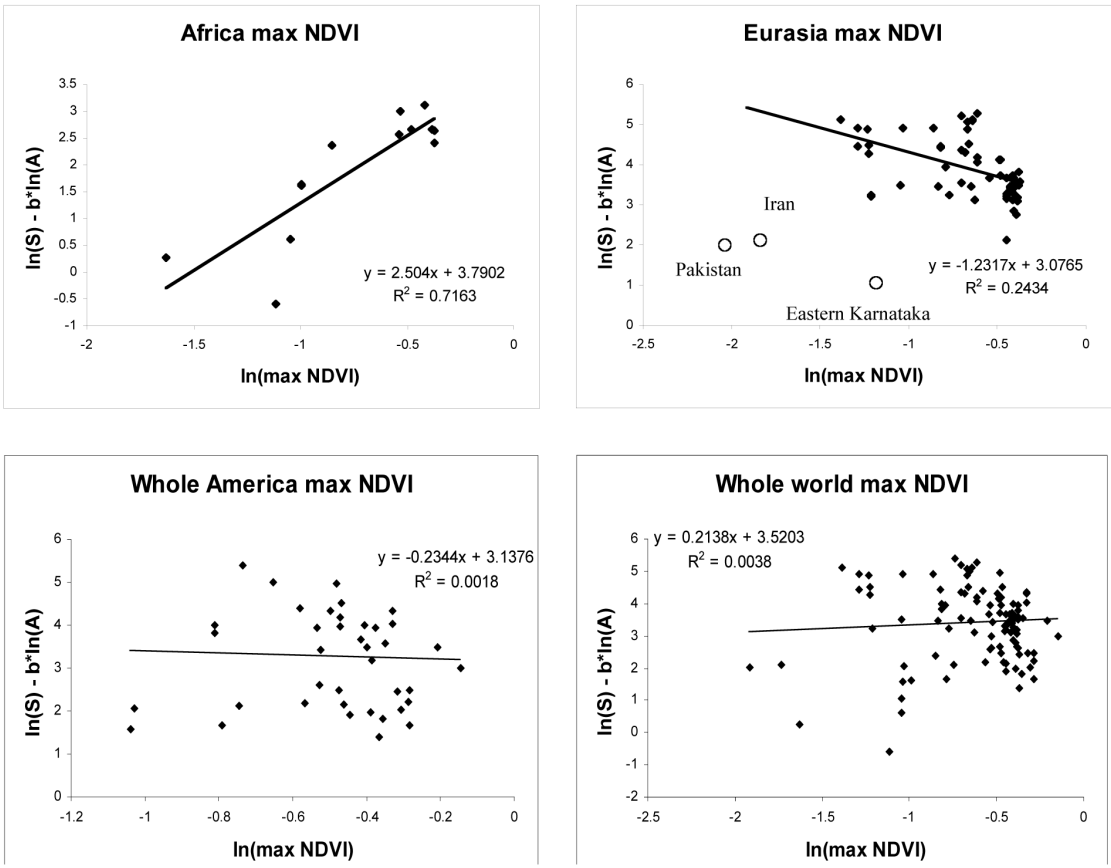

FIGURE 2. Relationship between logarithmically transformed maximum NDVI and species richness per unit area.

in the data set for the whole world to demonstrate the decrease of species richness from the tropics to the poles.

LITERATURE CITED

Brown, J.H. 1981. Two decades of homage to Santa-Rosalia: toward a general theory of diversity. Am. Zool. 21: 877-888.

Brown, J.H. & D.W. Davidson. 1977. Competition between seed-eating rodents and ants in desert ecosystems. Science 196: 880-882.

Cohen, S. B. (ed.) 1998. The Columbia Gazetteer of the World. Columbia University Press.

Connell, J.H. & E. Orias. 1964. The ecological regulation of species diversity. Am. Nat. 98: 399–414.

Connor, E.F. & E.D. McCoy. 1979. The statistics and biology of the species-area relationship. Am. Nat. 113: 791-833.

Currie, D.J. 1991. Energy and large-scale patterns of animal and plant species richness. Am. Nat. 137: 27-49.

Currie, D.J. & V. Paquin. 1987. Large-scale biogeographical patterns of species richness of trees. Nature 329: 326-327.

Francis, A.P. & D.J. Currie. 2003. A globally consistent richness-climate relationship for angiosperms. Am. Nat. 161: 523-536.

Gaston, K.J. 2000. Global patterns in biodiversity. *Nature* 416: 427-429.

Guegan, J.F., S. Lek & T. Oberdorff. 1998. Energy availability and habitat heterogeneity predict riverine fish diversity. Nature 391: 382-384.

Hutchinson, G.E. 1959. Homage to Santa Rosalia, or why are there so many kinds of animals? Am. Nat. 93: 145–159.

Kaspari, M., S. O'Donnell & J.R. Kercher. 2000. Energy, density, and constraints to species richness: ant assemblages along a productivity gradient. Am. Nat. 155: 280-293.

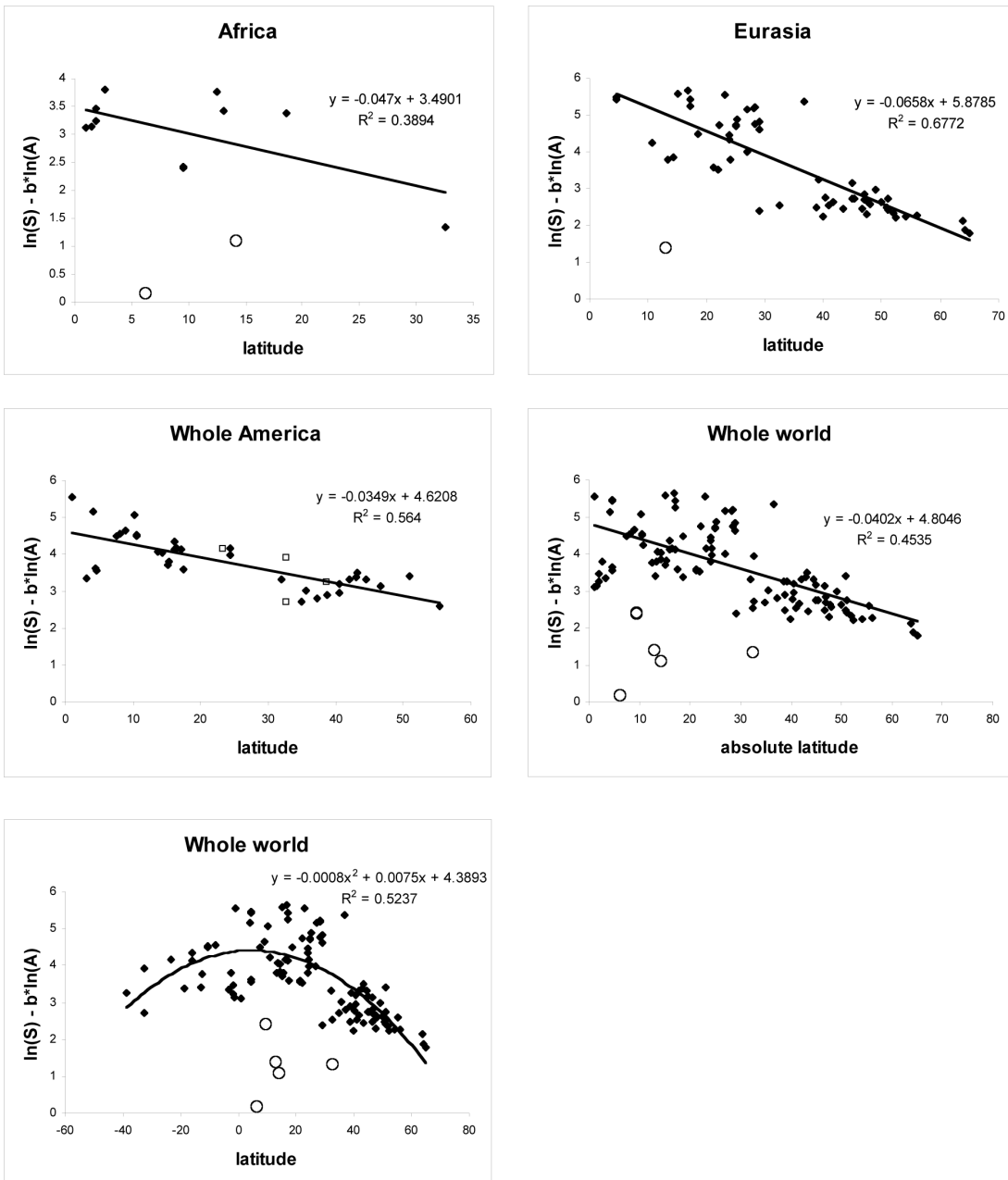

FIGURE 3. Relationship between latitude and logarithmically transformed species richness per unit area.

Kaspari, M., M. Yuan & L. Alonso. 2003. Spatial grain and the causes of regional diversity gradients in ants. Am. Nat. 161: 459-477.

Kerr, J.T. & M. Ostrovsky. 2003. From space to species: ecological applications for remote sensing. Trends Ecol. Evol. 19: 299-305.

Lieth, H. 1975. Modeling the primary productivity of the world. Pp. 237–263 *in*: H. Lieth& R.H. Whittaker (eds.), Primary Productivity of the Biosphere. Springer-Verlag, New York.

Rosenzweig, M.L. 1968. Net primary productivity of terrestrial communities: predictions from climatological data. Am. Nat. 102: 67–74.

Srivastava, D.S. & J.H. Lawton. 1998. Why more produc-

tive sites have more species: an experimental test of theory using tree-hole communities. Am. Nat. 152: 510-529.

Ustin, S.L., C.A. Wessman, B. Curtiss, E. Kasischke, J. Way & V.C. Vanderbilt. 1991. Opportunities for using the EOS imaging spectrometers and synthetic aperture radar in ecological models. Ecology 72: 1934–1945.

Wright, D.H. 1983. Species-energy theory: an extension of species-area theory. Oikos 41: 496-506.

Wright, D.H., D.J. Currie & B.A. Maurer. 1993. Energy supply and patterns of species richness on local and regional scales. Pp. 66-74

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MYCORRHIZAL DIVERSITY OF AN ENDEMIC TERRESTRIAL ORCHID

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KEY WORDS: Ascomycetes, Basidiomycetes, *Pinus radiata*, *Arctostaphylos, Piperia yadonii*

Mycorrhizal associations of the Orchidaceae - the largest and most diverse angiosperm family with an estimated number of taxa ranging between 17,000 and 35,000 – are highly complex. Plant-fungus interactions in this family can range from obligate- or facultative mycotrophy, mutualism, to minimal dependence on fungi in adulthood (Smith & Read 1997, Sivasithamparam *et al*. 2002, Taylor *et al.* 2002, Cameron *et al*. 2006). Terrestrial orchids also generally exhibit high habitat specificity. A unique ecological reliance upon mycorrhizal fungi is one of the reasons for habitat specificity in orchids. The fungal association is especially critical during the seed germination phase, but continues through the life-cycle of the plants, especially in the obligate mycotrophic species. It can therefore be expected that recruitment and persistence of orchids in their natural habitat depends on the availability of suitable fungi, which also form an underground network that connects orchids with the surrounding vegetation. Fungal ecology often is overlooked in orchid conservation projects, but if orchid-fungus relationships are very specific, the persistence of a population and/or success of a reintroduction program will be affected greatly by the presence or absence of suitable fungi in the soil, and by the habitat's ability to continuously support these fungi.

Piperia yadonii R. Morgan & J. Ackerman (a photosynthetic terrestrial) is endemic to Monterey County in California and is listed endangered by the US Fish and Wildlife Service. The Monterey area is part of the California Floristic Province Biodiversity Hotspot and represents the northern or southern limit of natural distribution of many plants, several of which co-occur with this orchid. *Piperia yadonii* occurs in two distinct habitats within its natural range: 1) lower elevation *Pinus radiata* D. Don forests with an herbaceous, sparse understory; and 2) higher elevation ridges in maritime chaparral growing beneath dwarfed *Arctostaphylos hookeri* G. Don shrubs. Biodiversity Hotspots are characterized both by exceptional levels of plant endemism and by dangerous levels of habitat loss. Only a few extant populations of *P. yadonii* remain and/or are known, and these are seriously threatened because of the increasing pressure from urban and recreational development in the area. One of the goals of USFWS is to downlist the species as threatened instead of delisting, primarily because its biology is not yet well enough understood to set delisting as an objective of the Recovery Plan (U.S. Fish and Wildlife Service 2004). Prior to this work, mycobionts of *P. yadonii* have not been studied. The specific objectives of this research were to: (1) identify fungal diversity associated with *P. yadonii* at selected sites/populations along two, a longitudinal and a latitudinal, transects across its natural distribution, and (2) identify how the fungal diversity of the orchid is related to both habitat types, and to the size of the orchid populations.

Materials and Methods

Roots of *Piperia yadonii* were collected between February and March of 2005 and 2006 from several locations across its natural distribution. Soil samples also were collected from each site to determine the soil chemical properties at each location. All materials were transported at 4^{o} C to the laboratory. Root sections were processed by using culture dependent and culture independent methods to assess the identity and diversity of the culturable and non-culturable mycobionts of *P. yadonii*. To obtain pure cultures of the peloton-forming fungi, we slightly modified the

method described in Sharma *et al*. (2003). When pure cultures of fungal isolates were obtained, DNA was extracted from these isolates. Fungal internal transcribed spacer (ITS-1) region then was amplified by using the primer pair ITS-1F/ITS-4 (Gardes & Bruns 1993). Digestion with restriction enzymes was used to obtain restriction fragment length polymorphism (RFLP) data. Based on the RFLP data, we subsequently selected isolates with unique profiles and subjected these to sequencing by using T7 and SP6 primers. For identifying the fungi by using culture independent method, thin sections of roots first were examined under the microscope to locate regions which contained fungal pelotons. We then extracted total DNA from these sections. In this case also, we amplified the fungal ITS-1 region by using the same primer pair as above, but followed with cloning the product. Clones were digested with *Eco*RV before selecting the unique profiles and subjecting these selected clones to sequencing with the same primers as above. In both cases, only those sequences whose T7 and SP6 (reverse complement) sequences matched completely were used further. Similar sequences were searched by using BLAST program (Altschul *et al*. 1997) by accessing the NCBI web-page (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). We used the default settings to conduct these searches.

Results and Discussion

Preliminary results show that fungi associated with *Piperia yadonii* primarily belong to the taxonomic groups Ascomycetes and Basidiomycetes, some of which have previously been reported as ectomycorrhizae of coniferous trees (Table 1). While some of the fungi identified thus far in our work have been reported from temperate terrestrial orchids, photosynthetic and non-photosynthetic, many have not previously been reported as orchid endophytes. Other fungi belonged to *Phomopsis* (Ascomycota), *Ceratobasidium* (Basidiomycota), *Neonectria* (Ascomycota), and *Phialocephala* (Ascomycota), which are reported to be coniferous ectomycorrhizae. *Ceratobasidium* spp., which are known to associate with many terrestrial orchids worldwide (Zettler *et al*. 2003), occurred at several sites including both *Pinus radiata* and *Arctostaphylos* spp. dominated habitats. Insofar, few individual species or taxonomic groups of fungi were observed at locations excepting MC (Table 1). This is a site which is close to urban areas and harbors a very small population of the orchid. On the other hand, the other sites, including those which harbor large numbers of orchids and/or are more isolated, yielded a low diversity of fungi associated with these plants. Because we are now using additional primers to amplify (in order to identify fungi unidentified this far) the fungal ITS-1 region of the same DNA samples, our forthcoming results may reveal a different pattern of fungal diversity in these locations.

While conserving plant diversity in biodiverse habitats is recognized as an outstanding global priority, there has been limited recognition of the macro level conservation in relation to ecosystem functioning at the micro level (Sivasithamparam *et al*. 2002). This is a result of a lack of knowledge and understanding of the micro level communities. Our results presented herein indicate that Ascomycetes, especially those forming ectomycorrhizae with the surrounding vegetation, may be equally critical to the persistence of this orchid as might be the saprophytic fungi or the Basidiomycetes from the *Rhizoctonia* complex, which are generally believed to be the primary orchid endophytes.

Literature Cited

Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller & D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389-3402.

Cameron, D.D., J.R. Leake & D.J. Read. 2006. Mutualistic mycorrhiza in orchids: evidence from plant-fungus carbon and nitrogen transfers in the green-leaved terrestrial orchid *Goodyera repens*. New Phytol. 171: 405-416.

Carter, J.P., J. Spink, P.F. Cannon, M.J. Daniels & A.E. Osbourn. 1999. Isolation, characterization, and avenacin sensitivity of a diverse collection of cereal-root-colonizing fungi. Appl. Environ. Microbiol. 65(8): 3364-3372.

Gardes, M. & T.D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113-118.

Julou, T., B. Burghardt, G. Gebauer, D. Berveiller, C. Damesin & M.A. Selosse. 2005. Mixotrophy in orchids: insights from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasonium*. New Phytol. 166 (2): 639-653.

Menkis, A., J. Allmer, R. Vasiliauskas, V. Lygis, J. Stenlid & R. Finlay. 2004. Ecology and molecular characterization of

TABLE 1. Fungal ITS-1 region of the mycobionts associated with roots of *Piperia yadonii*, an endemic terrestrial species, was sequenced and matched with previously known, similar sequences by using the BLAST program (Altschul *et al*. 1997) by using the NCBI web-page (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Previously Listed Source/Host	Closest sequence(s) found in GenBank by BLAST	Taxonomic Classification	Reference
MC[Z] Pine O-horizon soil	Uncultured ascomycete	Eukaryota; Fungi; Ascomycota	O'Brian *et al*. 2005
Picea abies decayed root	*Phomopsis columnaris*	Eukaryota; Fungi; Ascomycota	Menkis *et al*. 2006
Isolated from roots of *Triticum* sp.	*Phomopsis* sp.	Eukaryota; Fungi; Ascomycota	Carter *et al*. 1999
Mycorrhizal root tips (host *Cephalanthera damasonium*)	Uncultured mycorrhizal ascomycete	Eukaryota; Fungi; Ascomycota	Julou *et al*. 2005
Cultured fungus from mycorrhizal hemlock root tip	*Phialocephala fortinii*	Eukaryota; Fungi; Ascomycota	Lim *et al*. unpubl. data
Tricholoma matsutake fairy rings in a natural *Pinus densiflora* forest	Uncultured ectomycorrhizal fungus	Eukaryota; Fungi	Lian *et al*. 2005 (published only in NCBI database)
Natural *Tuber magnatum* truffle-ground	Uncultured Nectriaceae	Eukaryota; Fungi; Ascomycota	Murat *et al*. 2005.
Pinus sylvestris decayed root	*Neonectria macrodidyma*	Eukaryota; Fungi; Ascomycota	Menkis *et al*. 2006
BC *Picea abies* wood disc	*Ceratobasidium* sp.	Eukaryota; Fungi; Basidiomycota.	Menkis *et al*. 2004
PQR Environmental sample	Uncultured ectomycorrhiza	Eukaryota; Fungi; Basidiomycota	Murat *et al*. 2005
SFB *Picea abies* wood disc	*Ceratobasidium* sp	Eukaryota; Fungi; Basidiomycota	Menkis *et al*. 2004
PL *Taxus chinensis* var. *mairei*	Fungal endophyte	Eukaryota; Fungi; Ascomycota	Wang & Wang Unpublished
Not listed	*Cercophora sparsa* voucher	Eukaryota; Fungi; Ascomycota	Miller & Huhndorf 2004
JP Soil	Uncultured soil fungus	Eukaryota; Fungi	Waldrop *et al*. unpubl. data
MP *Picea abies* wood disc	*Ceratobasidium* sp.	Eukaryota; Fungi; Basidiomycota	Menkis *et al*. 2004

[Z]MC, BC, PQR, SFB, PL, JP, and MP are codes for locations from which roots of *Piperia yadonii* were collected.

dark septate fungi from roots, living stems, coarse and fine woody debris. Mycol. Res. 108: 965-973.

Menkis, A., R. Vasiliauskas, A.F.S. Taylor, E. Stenstrom, J. Stenlid & R. Finlay. 2006. Fungi in decayed roots of conifer seedlings in forest nurseries, afforested clear-cuts and abandoned farmland. Plant Pathol. 55(1): 117-129.

Miller, A.N. & S.M. Huhndorf. 2004. Using phylogenetic species recognition to delimit species boundaries within Lasiosphaeria. Mycologia 96(5): 1106-1127.

Murat, C., A. Vizzini, P. Bonfante & A. Mello. 2005. Morphological and molecular typing of the below-ground fungal community in a natural *Tuber magnatum* truffle-ground. FEMS Microbiol. Lett. 245(2): 307-313.

O'Brien H.E., J.L. Parrent, J.A. Jackson, J.M. Moncalvo & R. Vilgalys. 2005. Fungal community analysis by large-scale sequencing of environmental samples. Appl. Environ. Microbiol. 71(9): 5544-5550.

Sharma, J., L.W. Zettler & J.W. Van Sambeek. 2003. A survey of mycobionts of federally threatened *Platanthera praeclara* (Orchidaceae). Symbiosis 34: 145-155.

Sivasithamparam, K., K.W. Dixon & R.L. Barrett. 2002. Microorganisms in plant conservation and biodiversity. Pp.

195–226. Kluwer, Dordrecht, The Netherlands.

Smith, S.E. & D.J. Read. 1997. Mycorrhizal symbiosis. Academic Press, New York, NY, USA.

Taylor D.L., T.D. Bruns, J.R. Leake & D.J. Read. 2002. Mycorrhizal specificity and function in myco-heterotrophic plants. Pp 375-414 *in*: R. Sanders, I.R. & M. van der Heijden (eds.). The ecology of mycorrhizas. Ecological Studies vol. 157. Springer-Verlag, Berlin, Germany.

U.S. Fish and Wildlife Service. 2004. Recovery Plan for Five Plants from Monterey County, California. U.S. Fish and Wildlife Service, Portland, Oregon. Xii+ 159 pp.

Zettler, L.W., J. Sharma & F.N. Rasmussen. 2003. Mycorrhizal Diversity. Pp. 205–226 *in*: Dixon, K.W., S.P. Kell, R.L. Barrett & P.J. Cribb (eds.). Orchid conservation. Natural History Publications, Kota Kinabalu, Sabah, Malaysia.

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DOES INTEGRATED CONSERVATION OF TERRESTRIAL ORCHIDS WORK?

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Introduction to integrated conservation

Effective plant conservation involves careful consideration and difficult choices when investing limited resources to conservation programs and policies. The conservation practice must integrate the understanding of existing and future environmental threats, taxonomic distinctiveness, numbers of individuals in populations, reproductive biology, *ex situ* propagation and the maintenance of evolutionary processes influencing population distribution patterns. For this to be possible, conservation should involve detailed experimentation directed at continued survival of the species in both an on site (*in situ)* ecological context and off site (*ex situ*) laboratory based context (Ramsay & Dixon, 2003). Thus the development of effective conservation strategies, must strike a balance between the need for urgent action to avoid further loss and the search for essential information and understanding of the species or ecosystem to be conserved. The integrated conservation strategy emphasizes the study of interactions among land conservation, biological management, *ex situ* research and propagation and (re)introduction and habitat restoration (Hopper, 1997). Integrated conservation approaches vary according to different species with their habitats and distribution characteristics; however the basic concept remains the same (Fig 1).

Orchid Conservation

Many orchids are characterised by a symbiotic relationship with a mycorrhizal fungus and variety of pollination syndromes (Le Tacon & Selosse 1994, Rasmussen 1995). The interaction of these attributes with often specialised habitat requirements has played a significant role in the evolutionary diversification and the present distribution of these flowering plants (Cozzolino & Wedmer 2005, Otero & Flanagan 2006).

To recover a rare or threatened orchid species in a timely and effective manner, an integrated conservation approach must be applied to the understanding of plant-fungus interactions, pollination syndromes, population genetic structure and evolutionary processes, *in situ* habitat requirements and *ex situ* conservation concepts. Many workers have effective-

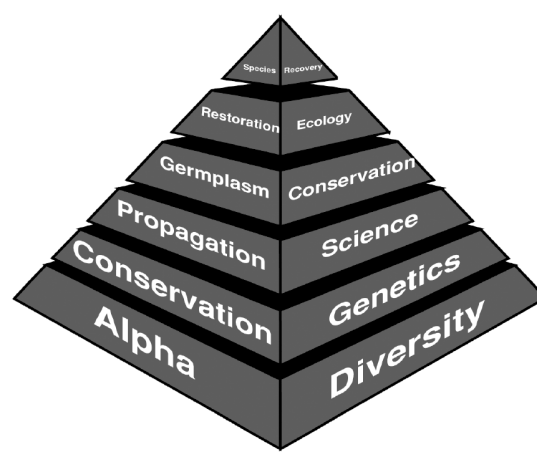

FIGURE 1. A pyramid model of the integrated conservation concept (Dixon & Batty 2003). Species recovery (top step) is an integrated process requiring all the five lower steps shown in this figure.

ly researched these principles as singular entities in a conservation context, however, over the last decade with the successful development of a raft of new conservation technologies can the integrated approach be adopted at a whole species level. The aim of this study is to test the principle of a science-based integrated conservation approach (Falk 1990) towards the recovery of nationally endangered orchid *Caladenia huegelii* H. G. Reichb. Key elements of the study include understanding the basis of rarity in the species, defining and abating threatening processes and developing translocation and management strategies to enhance species resilience and persistence in extant locations.

Caladenia huegelii occurs from Perth to Busselton in Western Australia along the Bassendean Sand geological system characterized by deep, highly infertile siliceous sands in species rich banksias woodland (Hoffman & Brown 1992). Extensive surveying of the all known populations and potential habitat for new populations over three successive growing seasons revealed that the species is significantly reduced to just 18 locations, seven of these are new populations and four of the 18 contain just one plant. Urban development contributed to the loss of populations and large-scale loss of suitable habitat for recruitment opportunities. Grazing of leaves and buds by caterpillars, tuber diggings by bandicoots and the grazing of developing seed capsules by kangaroos were observed as other threatening processes and key management issues.

Population Genetics

Conservation strategies for the maintenance of genetic diversity rely greatly on detailed study of the genetic structure, demographic history and evolutionary potential of plant populations (Fay & Krauss 2003). To develop an understanding of the genetic structure and variability of *C. huegelii* populations, microsatellite markers were used to screen 460 samples representing all populations over its geographical range for polymorphism across seven loci. Genetic divergence among populations was correlated with geographical distance investigating genetic isolation on both population and regional levels. In results comparable to another sexually deceptive *Caladenia* species, *C. huegelii* exhibited similar low levels of genetic differentiation (Fst = 0.102), with 90% of genetic variation partitioned within populations (Peakall & Beattie 1996).

Fungal Associations

Fungal associates of *Caladenia* species show a distinctively fragmented distribution in the landscape, a feature that may be contributing to the rarity of these orchids (Batty *et al*. 2001a, Brundrett *et al*. 2003). Working with *C. huegelii* and common sympatric congeners, we investigated the diversity and specificity of associated endophytes isolated from adult plants over the geographical range of the orchid through large scale *in situ* and *in vitro* matrix germination experiments. These studies demonstrated a highly specific plant-endophyte relationship in *C. huegelii* indicating that orchid distribution may be limited to the type and availability of a specific symbiont at a site, a possible driver of rarity in this species. Endophytes isolated from *C. huegelii* adult plants over the range of the taxon and germinated *in situ* protcorms were found to effectively germinate seed from across the habitat range of the orchid. No significant home site advantage was observed for orchid seed germinating on an endophyte collected from the same plant. Common congeneric species utilized a range of available endophytes in both *in situ* and *in vitro* germination trials including the *C. huegelii* fungus suggesting potential implications for competitive niche occupancy.

The results of high specificity of *C. huegelii* endophytes were reinforced by sequencing the highly variable ITS region of 50 endophytes isolated from both adult plants of *C. huegelii* and *in situ* protocorms, using the fungal specific primers ITS1 and ITS4, finding identical sequences of all endophytes sequenced. Further sequencing of endophytes isolated from common congeners revealed sequence variation when analyzed with those from *C. huegelii* providing additional support to germination results. Analysis of the ITS sequences isolated from the range of *Caladenia* species showed high similarities with a sequence from a *Sebacina vermifera* originally isolated from *Caladenia dilatata* (Bougoure *et al*. 2005) and matching sexual stages identified by Warcup (1971) for *Caladenia*.

Pollination Syndrome

This species adopts a sexually deceptive pollination syndrome employing male thynnid wasps for the transfer of pollen from one plant to another (Stoutamire 1983). We investigated the natural abundance of the specialized wasp pollinators at the key study site at Ken Hurst Park site using the established 'baiting' techniques used in sexual deceptive systems. A detailed study of the natural pollination success for *C. huegelii* showed for the species a low rate of seed set with <4% of flowering plants producing seed in both the 2005 and 2006 seasons. The study compared the success to other sexually deceptive pollinated orchids and showed that *C. huegelii* is on the lower end of the pollination success scale compared with related genera such as *Drakaea* where pollination success rates can be in excess of 80% (R. Phillips, pers. comm. 2006) and was very low compared to most other sexually deceptive orchids with a mean pollination rate of 33.1% (Tremblay *et al*. 2005, J. See, pers. comm). All baiting trials indicated an absence of the pollinator at the site based on the sampling method used. Comparison with pollination success in other *Caladenia* species showed the low levels of pollination recorded in *C. huegelii* fall within the pollination range for *Caladenia* indicating that the low seed set in *C. huegelii* is not unexpected. Clearly to sustain suitable seed output from wild and reintroduced populations of the orchid, there may be either the need to increase pollinator abundance or have intervention management involving artificial pollination.

Reintroduction and transplanting

Protocorms generated from germination experiments were used in subsequent pot and field trials to optimize tuberisation in developing seedlings thus increasing the transfer success of plants *in situ*. The reintroduction of orchids to field sites was markedly enhanced by outplanting two-year old seedlings over one-year old seedlings with irrigation and protective cages enhancing plant growth and overall survival. Out-plants were monitored fortnightly over the 2006 growing season and 100% survival was observed for two-year old seedlings compared to 5% survival in the seedlings out-planted in their first season of growth. Increased survival and seedling growth was observed in seedlings outplanted in close proximity to adult plants in comparison with artificially inoculated substrate and the no inoculum control further reinforcing endophytic potential for reintroduction success.

Ex situ conservation

Although *in situ* conservation of threatened taxa takes precedence in management priorities, the requirement for *ex situ* conservation and storage of germplasm is increasingly recognized as an imperative tool in the preservation and maintenance of biodiversity (Batty *et al.* 2001b, Fay & Kruass, 2003). Using the results of the molecular study to determine significant populations and the methods of Batty *et al* (2001b) to ensure long term survival of stored germplasm, a representative sample of viable *C. huegelii* seed was placed in liquid nitrogen (-196°C). Although no significant sequence variation of *C. huegelii* associated endophytes was observed, a range of isolates that best promoted seed germination across all populations have also been successfully preserved in liquid nitrogen.

Conclusion

This study has demonstrated that within a relatively short time frame it is possible to develop a comprehensive and focused data set leading to effective integrated conservation. Using seedling establishment in the wild as a key benchmark of success the study results provided confidence that recovery operations can be effectively undertaken if there is an understanding of:

- Mycorrhizal specificity
- Biogeographic variation in mycorrhiza
- Genetic diversity of plants and fungi
- Seed banking and endophyte banking
- Pollination ecology.

The challenge now is to adopt and test these key principles in terms of multi-species recovery operations for orchids possibly starting with congeneric taxa and then working on phylogenetically related taxa. The future of orchid conservation depends on effective and timely delivery of orchid conservation where there are active links between on-site conservation actions and research programs.

LITERATURE CITED

Batty A.L., K.W. Dixon, M. Brundrett & K. Sivasitthampara. 2001a. Long-term storage of mycorrhizal fungi and seed as a tool for the conservation of endangered Western Australian terrestrial orchids. Austral. J. Bot. 49: 1 - 10.

Batty A.L., K.W. Dixon, M. Brundrett & K. Sivasitthampara. 2001b. Constraints to symbiotic germination of terrestrial orchid seed in a Mediterranean bushland. New Phytol. 152: 511 - 520.

Bougoure J.J., Bougoure D.S., Cainey J.W.G. & Dearnaley J.D.W. (2005) ITS – RFLP and sequence analysis of endophytes from *Acianthus, Caladenia* and Pterostylis (*Orchidaceae*) in southeastern Queensland. Mycol. Res. 109(4): 452 – 460.

Brundrett M.,A. Scade, A.L. Batty, K. Dixon & K. Sivasithampara. 2003. Development of in situ and ex situ seed baiting techniques to detect mycorrhizal fungi from terrestrial orchid habitats. Mycol. Res. 107: 1210-1220.

Cozzolino S. & A. Widmer. 2005. Orchid diversity: an evolutionary consequence of deception? Trends Ecol. Evol. 20: 487-494.

Dixon K.W. & A.L. Batty. 2003. Integrated conservation of Australian orchids - a local and global perspective. In 'Orchid Conservation Forum II'. Royal Botanic Gardens, Melbourne.

Falk D.A. 1990. Integrated strategies for conserving plant genetic diversity. Ann. Missouri Bot. Gard. 77: 38-47.

Fay M.F. & S.L. Krauss. 2003. Orchid conservation genetics in the molecular age. Pp. 91-112 *in*: K.W. Dixon, S.P. Kell, R.L. Barrett & P.J. Cribb (eds.). Orchid Conservation. Natural History Publications (Borneo): Kota Kinabalu, Sabah, Malasia.

Hoffman N. & A. Brown. 1992. Orchids of South-West Australia, 2nd Edition. University of Western Australia Press, Nedlands.

Hopper S.D. 1997. An Australian perspective on plant conservation biology in practice. Pp. 255-278 *in*: P.L. Fiedler & P.M Kareiva (eds.). Conservation Biology for the Coming Decade. Chapman and Hall: New York.

Le Tacon F. & M.A. Selosse 1994) The mycorrhizal symbiosis in the evolution and the colonization of continents by the life. [French]. Acta Bot. Gallica 141: 405-419.

Otero J.T. & N.S. Flanagan. 2006. Orchid diversity – beyond deception. Trends Ecol. Evol. 21 : 64-65.

Ramsay M.M. & K.W. Dixon. 2003. Propagation science, recovery and translocation of terrestrial orchids. Pp. 259-288 *in*: K.W. Dixon, S.P. Kell, R.L. Barrett & P.J. Cribb (eds.). Orchid Conservation. Natural History Publications (Borneo): Kota Kinabalu, Sabah, Malasia.

Rasmussen H.N. 1995. Terrestrial Orchids, from Seed to Mycotrophic Plant. Cambridge University Press: Cambridge.

Stoutamire W.P. 1983. Wasp-pollinated species of *Caladenia* (Orchidaceae) in South-western Australia. Austral. J. Bot. 31: 383-94.

Tremblay R.L., J.D. Ackerman, J.K. Zimmerman & R.N. Calvo. 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. Bot. J. Linn. Soc. 84: 1-54.

Warcup J.H. 1971. Specificity of mycorrhizal association in some Australian terrestrial orchids. New Phytol. 70: 41-46.

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EVOLUTION IN SMALL POPULATIONS: EVIDENCE FROM THE LITERATURE AND EXPERIMENTAL RESULTS

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The great taxonomic diversity of the Orchidaceae is often attributed to adaptive radiation for specific pollinators driven by selection for outcrossing. However, when one looks beyond the product to the process, the evidence for selection is less than overwhelming. Here we review a number of investigations that attempted to determine if natural selection is present in a variety of orchids based on the data from the literature in addition to our own research to understand the relative importance of this process. We illustrate through these examples that attempts to measure and demonstrate evidence for natural selection reveal selection coefficients that are most often small and non-significant. From the literature we show selection studies for morphological variation in *Lepanthes rupestris* Stimson (Cintrón-Berdecia & Tremblay 2006), unpublished work on selection coefficients for color variation in *Lepanthes rupestris* (Tremblay & Ackerman, submitted), color variation in *Bletia patula* Graham (Ackerman & Carromero 2005) and in *Psychilis monensis Sauleda* (Aragon and Ackerman 2004), and phenotypic selection for size in *Caladenia gracilis* R. Br.

Prior to any studies evaluating the potential for evolution, it is important to determine that the characteristics of interest are heritable. Genetic variation is not the only cause of morphological variation. Morphological variation can be a result of the environmental factors and also by the interaction between genetics and environmental (G X E, phenotypic plasticity). In the studies that follow we assume that most morphological variation is a direct result of genetic variation or genetic by environment interaction and not dominated by environment. Previous results suggest that significant variation attributable to environment can occur in some floral characteristics but others appear relatively unaffected by environmental changes (Morales Vargas 2003).

Size: Is it superficial?

In the first example Cintrón-Berdecia and Tremblay (2006) sought to identify floral characters that suggested evidence for either linear, disruptive or stabilizing selection on flowers of more then 200 individuals of *Lepanthes rupestris* Stimson in seven populations along two river systems. None of the analyses suggested that any of the characters were under selection in all populations. They found scattered evidence that some of the characters had positive reproductive output through either pollinaria removal or fruit set when the floral character was larger (length of column; in two populations; mid lobe length, lip width, posterior petal lobe), while other characters suggested negative effect when characters were larger (width of anterior petal lobe, front lip length, flower size). A few characters appear to be subjected to disruptive selection (length of anterior petal lobe, width of anterior petal lobe, front lip width) and stabilizing selection (length posterior petal lobe, distance between sepals, Table 1). In spite of this the most outstanding result of the survey is the complete inconsistency in significant selection coefficients in any of the characters among populations. The character with the most frequent significant selection coefficient was the length of the column, which was found only in two populations, through both pollinaria removal and fruit set.

When all data from the seven populations are summed and analyzed, the length of the column is again the only character where the linear selection coefficient is significantly different using both measure of reproductive success (larger column length have higher selection coefficient on reproductive success, 6.2% (pollinaria removal) and 5.3% (fruit set). The length of the lip and distance between sepals were also found to be positively correlated with size through pollinaria removal.

TABLE 1: Univariate (*s'*, *ß''*) and multivariate (*γ'*) selection acting through female success (fruit set) fitness on 12 characteristics in one populations of *Lepanthes rupestris* and the sum of all seven populations (populations 2 to 7 not shown; see Cintrón - Berdecia and Tremblay 2006). Standardized selection coefficients are represented as directional (*s', ß'*) and non-lineal (*γ'*, negative values = stabilizing selection, positive values = disruptive selection) and given in units of phenotypic standard deviation. Bold numbers are significant at the following levels, *P £ 0.05, **P £ 0.

Female Fitness	Population 1				All Populations			
Traits	n	*s'*	*ß'*	*γ'*	n	*s'*	*ß'*	*γ'*
Length of Column	35	**.189***	.200	.054	201	**.062****	.048	.001
Width Sepal Dorsal	29	.163	.058	-.054	189	.022	-.018	-.001
Length Posterior Petal Lobe	29	.113	-.066	-.141	187	.018	.002	-.001
Width Posterior Petal Lobe	29	.086	.032	-.077	188	.029	.032	-.018
Length Anterior Petal Lobe	29	.131	.043	-.123	189	.044	-.040	-.001
Width Anterior Petal Lobe	29	.218	.122	-.003	189	.010	.058	.003
Front Lip Length	29	.077	.061	-.044	186	**.052****	.043	-.003
Front Lip Width	29	**.439***	.182	.036	186	.012	.009	.011
Mid Lobe Length	26	-.033	-.200	.032	176	.023	.023	-.002
Anther Cap Open	28	-.068	-.204	.043	184	.016	-.092	.011
Distance Between Sepals	27	.040	.058	.008	184	.017*	.007	011
Flower Size	33	.090	.170	-.158	208	.010	-.004	.008

However, fruit set suggests that the distance between sepals was under disruptive selection (Table 1).

Color: Can you see it and do you care?

In the second example Tremblay and Ackerman (unpublished) sought to measure phenotypic selection on petal color variation in populations of *Lepanthes rupestris*. Plants have flowers with either unicolor or bicolored petals. The two color morphs are otherwise inseparable morphologically. Reproductive success is skewed towards few individuals and effective population sizes are estimated to be small. We censused seven populations monthly for 20 months or more and noted flower production and petal color pattern. Each flower was checked for pollinarium removal (a measure of male fitness) and fruit production (a measure of female fitness). In all populations, plants with bicolored petals dominated, comprising 63-82% of individuals. Nevertheless the two types were indistinguishable based on reproductive success. Flower color pattern was generally not associated with either male or female reproductive success within or among populations or over time. Although we were unable to tag fitness to petal color patterns, the consistent ratio of color morphs among populations suggests that factors other then just drift are responsible for the frequencies we observed within a population.

In food-deceptive orchids, variation in floral characteristics associated with pollinator attraction is expected to be high, largely due to either relaxed selection or negative frequency-dependent selection (Ackerman & Galarza-Pérez 1991). Relaxed selection would occur if flower color made no difference, as may be the case for *Lepanthes rupestris*. Negative frequency dependent selection occurs when being different imparts an advantage because pollinators will more quickly learn that similar phenotypes have no rewards. Ackerman and Carromero (2005) surveyed populations of *Bletia patula* in the Dominican Republic in a region where two color morphs were common: pink flowered and white flowered plants. While no morphological differences between the two color morphs were detected, there were differences in reproductive success between the two. The white-flowered plants had an advantage through male function (they were more likely to have pollinaria removed than pink-flowered plants). However, success was not related to color morph frequencies, neither negatively nor positively. The two color morphs also occur in Puerto Rico, but the white-flowered plants are extremely rare precluding any comparison between populations of the two islands.

When color variation is bimodal, such as in *Lepanthes rupestris* and *Bletia patula*, it is relatively easy to associate a color morph with pollinator behavior and reproductive success. However, in most orchids with substantial variation in floral color the variation is continuous. Under such circumstances it is somewhat more difficult to interpret variation in pollinator behavior and plant reproductive success. In an experimental study Aragón and Ackerman (2004) manipulated color variation in *Psychilis monensis*, creating uniformly and variably colored populations. As plants of this species flower all year, treatments were rotated among populations over time. They found that over 50% of the variation in either male or female reproductive success was explained by time and site with no significant effect of treatment except as part of a three-way interaction of time X site X treatment. Population variation in floral color had little or no effect on reproductive success. Major community changes had occurred during the experiment with flowering activity of sympatric species falling dramatically and by the third run of the experiment, only *P. monensis* was in flower. This is when the number of effective visits significantly increased. They concluded that high natural levels of color variation may be more influenced by drift than selection

Showing off: Size matters

Selection may also act on floral display and thus there is an opportunity for pollinator-mediated selection (Williams & Conner 2001, Kobayashi *et al*. 1997, Worley *et al*. 2000, Totland *et al*. 1998). *Caladenia (Stegostyla) gracilis* R. Br. is a widespread terrestrial orchid from Eastern Australia. Tremblay (2006) surveyed populations at two sites in the state of Victoria.

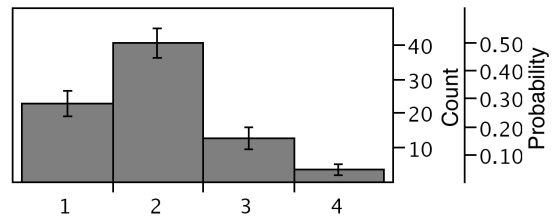

FIGURE 1: Frequency (s.e.) distribution of floral display size in 81 individuals of *Caladenia gracilis* at two sites in the state of Victoria, Australia.

Inflorescences have 1-6 flowers, each about 30 mm across (Tremblay 2005). A total 81 plants at two sites were sampled for reproductive success (pollinaria removed, pollination and fruit set) as a function of number of flowers on a plant. Plants with two flowers was the most common display size at both sites (mean + s.e.; 2.0 ± 0.11; Figure 1).

Plants that have fewer flowers have a very low probability of having their pollinaria removed or deposited or setting fruit. The probability of reproductive potential was significantly higher in multi-flowered individuals (Logistic regression; log likelihood = 8.134, df 1, 80, $p < 0.0001$: Table 2).

A literature review of some of the evidence of the effect of floral display on reproductive success in orchids suggests that when significant effects occur, reproductive success is higher in larger inflorescences, although it does not necessarily increase proportionately with an increase in the number of flowers (Montalvo & Ackerman 1987). However, in a number of orchid species pollinators appear to have no preference for floral display size (Table 3). The ability to detect a significant effect is sample size dependent, however there is no evidence to suggest that these non-significant results are an aberration. Moreover, the effect of reproductive success on floral display can be

TABLE 2. The expected male and female reproductive success of individuals of *Caladenia gracilis* with varying floral display. Analysis of receiver operating characteristics (ROC) calculates the most likely state of each of the type of floral display from the logistic regression equation. Thus a flower on a two-flowered inflorescence has a 32% chance of having the pollinaria removed, while on a four-flowered inflorescence, an individual flower has an 89% chance of having the pollinaria removed.

Floral display (number of flowers)	Expected percent pollinaria removal	Expected percent pollinaria deposition	Expected percent Fruit set
1	0.11	0.09	0.10
2	0.32	0.17	0.21
3	0.66	0.30	0.37
4	0.89	0.47	0.57

inconsistent among time and space, as it has been shown for other characters (Maad 2000, Ehlers *et al.* 2002; Tremblay & Ackerman, unpublished).

Among the different studies discussed above, no general pattern of selection was observed for color morphs or morphological characters. The selective advantage of floral color could be affected by a number of variables, the first is it assumes that the pollinators can visualize the color variation perceived by humans, secondly it assumes that the color variation is sufficiently discrete so they care enough to make a choice between the variants. Furthermore, the ecological context may make all the difference as well. Some European, rewardless orchids have dramatic flower color polymorphisms as in *Bletia patula*, but unlike the tropical species, color and its frequency have been shown to make a difference to orchid reproductive success (Smithson & Macnair 1997, Gigord *et al*. 2001). Such differences in response to color poly-

TABLE 3: Effect of floral display on reproductive success in orchids. "+" = positive effect of larger floral display on reproductive success, "-" = negative effect of larger floral display on reproductive success, "D" = disruptive selection, NS = no significant effect of floral display on reproductive success.

Species	**Variation in number of flowers**	**Pollinaria removal**	**Fruit set**	**References**
Aspasia principissa Rchb. f.	1-7	+	+	Zimmerman & Aide 1989
Brassavola nodosa (L.) Lindl.	1-5	+	+	Schemske 1980; Murren & Ellisson 1996
Calopogon tuberosus (L.) Britton, Sterns & Poggenb.	1-10		NS	Firmage and Cole 1988
Comparettia falcata Poepp. & Endl.	1-9	+	+	Rodríguez *et al.* 1992
Cyclopogon cranichoides (Griseb.) Schltr.	8 - > 40		+	Calvo 1990
Dactylorhiza maculata (L.) Soó	Mean 15		NS	Vallius 2000
Elythranthera brunonis (Endl.) A.S. George		NS	NS	Tremblay *et al.* this issue
Psychilis krugii (Bello) Sauleda	1-8	NS	NS	Ackerman 1989
Epidendrum exasperatum Rchb. f.	6-358	+		Calvo 1990
Epipactis helleborine (L.) Crantz	15-30? data absent from paper	+	+	Ehlers, *et al.* 2002,Piper and Waite 1988
Gastrodia exilis Hook. f.	2-13		NS	Pedersen *et al.* 2004
Ionopsis utricularioides (Sw.) Lindl.	1-44	+	+(-)*	Montalvo & Ackerman, 1987
Lepanthes wendlandii Rchb. f.	1-123		NS	Calvo 1990
Malaxis massonii (Ridl.) Kuntze	6-106	+	+ year dependent	Aragón & Ackerman 2001
Oeceoclades maculata (Lindl.) Lindl.	4-16		NS	Calvo 1990
Orchis purpurea Huds.	9-98		NS	Jacquemyn *et al.* 2002
Platanthera bifolia (L.) Rich.	10-20	+	+	Maad 2000
Oligochaetochilus (Pterostylis) longifolia (R. Br.) Szlach.	1-5 data absent from paper	NS	NS	Hamilton 2003
Rhyncholaelia glauca (Lindl.) Schltr.	1-17	NS	NS	Flores-Palacios & Garcia-Franco 2003
Tolumnia variegata (Sw.) Braem	1-15		D	Sabat & Ackerman 1996

* Large inflorescences produce more fruits than small inflorescences, but for those plants that produce fruit, there is a strong *negative* relationship between fruit number and flower number.

morphisms among species with similar pollination strategies suggests that other factors are at play.

However consistency in selection of larger inflorescence appears to be common. Selection on floral display size and reproductive successes clearly shows that larger inflorescences size appears to offer an advantage. However, the observed advantage may not be easily explained. Tremblay (2006) showed that larger display sizes are advantageous in *Caladenia gracilis,* which was not expected, as this species belongs to a mostly single flowered clade of the Caladeniinae. If this evolutionary advantage has been present prior to the study, then why are larger inflorescences not more common in this clade? Constraints to display size and its evolution must be present and could be influenced by complex heritability, inbreeding depression, costs to reproduction and phenotypic plasticity. Behavioral differences among pollinators of the different species may also play a role as would the ecological context in which the orchid populations exist. To evaluate the potential for natural selection on floral display a number of parameters need to be evaluated. Do flowering individuals come back with the same number of flowers (at least in the same range) as previous flowering events? Is the lifetime reproductive success of a large individual equal to small individuals? What is the importance of phenotypic plasticity on floral display? At present those data are generally missing from the literature for terrestrial and epiphytic orchids.

We found no evidence in the orchid literature on the frequency of individuals expressing a specific floral display size among flowering period. Determining if that display size is genetically influenced, however a substantial amount of evidence suggests that there is a cost to reproduction in many species of orchids which often results in reduce display size in the next reproductive bout or emerging as vegetative individual or not at all (dormant) (Pfeifer *et al*. 2006, Coates *et al*. 2006 Tremblay *et al*. 2005). Consequently display size may be very plastic and not a good character for predicting selection coefficients. Short surveys maybe inconsequential to evolutionary processes. It may be more appropriate to evaluate this character considering the lifetime reproductive success of the individuals and a measure of mean display size throughout that lifespan.

Gentry and Dodson (1987) have suggested that evolutionary processes in orchids can be quick and that within a few generations cladogenesis can occur. Under a basic Darwinian evolutionary process selection coefficients would then have to be high and consistent among time periods for evolution to be quick. Others contend that such processes may be slow (Soto-Arenas 1996) and there is some genetic evidence for it (Corrias *et al*. 1991, Rossi *et al*. 1992, Ackerman & Ward 1999). A third possibility is that in many situations, genetic drift may be equally important as natural selection in fostering genetic and morphological variation in this family (Tremblay *et al.* 2005). We favor this vision and suggest that the great diversity in this family to be largely a consequence of sequential and rapid interplay between drift and natural selection.

LITERATURE CITED

Ackerman J. D. & W. Carromero. 2005. Is reproductive success related to color polymorphism in a deception-pollinated, tropical terrestrial orchid? Caribbean J. Sci. 41: 234-242.

Ackerman, J. D. & S. Ward. 1999. Genetic variation in a widespread, epiphytic orchid: where is the evolutionary potential? Syst. Bot. 24: 282-291.

Aragón, S. & J. D. Ackerman. 2001. Density effects on the reproductive success and herbivory of *Malaxis massonii*. Lindleyana 16: 3-12.

Calvo, R.N. 1990. Four-year growth and reproduction of *Cyclopogon cranichoides* (Orchidaceae) in South Florida. Amer. J. Bot. 77: 736-741.

Cintrón-Berdecía S.-T. & R. L. Tremblay. 2006. Spatial variation in phenotypic selection on floral characteristics in an epiphytic orchid. Folia GeoBot. 41: 33-46.

Coates, F., I. Lunt & R. L. Tremblay. 2006. The effect of fire, rainfall, herbivory on population dynamics of a threatened terrestrial orchid from south-eastern Australia, *Prasophyllum correctum* D.L. and consequences for management. Biol. Conserv. 129: 59-69.

Corrias, B., W. Rossi, P. Arduino, R. Cianchi & L. Bullini. 1991. *Orchis longicornu* Poiret in Saardinia: genetic, morphological and chorological data. Webbia 45: 71-101.

Ehlers, B.K., J.M. Olesen & J. Agren. 2002. Floral morphology and reproductive success in the orchid *Epipactis helleborine*: regional and local across-habitat variation. Pl. Syst. Evol. 236: 19-32.

Firmage, D.H. & F.R. Cole. 1988. Reproductive success and inflorescence size of *Calopogon tuberosus* (Orchidaceae). Amer. J. Bot. 75: 1371-1377.

Flores-Palacios, A. & J.G. Garcia-Franco. 2003. Effects of floral display and plant abundance on fruit production of *Rycholaelia glauca* (Orchidaceae). Rev. Biol. Trop. 51: 71-78.

Gentry, A. L. & C.H. Dodson. 1987. Diversity and biogeography of neotropical vascular epiphytes. Ann. Missouri Bot. Gard. 74: 205-233.

Gigord, L. D. C., M. Macnair & A. Smithson. 2001. Negative frequency-dependent selection maintains a dramatic flower color polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soó. Proc. Natl. Acad. Sci. USA 98: 6253-6255.

Hamilton, J. 2003. Towards an understanding of pollination in the Australian Terrestrial orchid *Pterostylis longifolia* (Brown). Honours thesis, The Australian National university, Camberra, Asutralia.

Jacquemyn, H., R. Brys & M. Herny. 2002. Flower and fruit production in small populations of *Orchid purpurea* and implications for management. Pp. 67-84 *in:* P. Kindlmann, J. H. Willems and D. F. Whigham (eds.). Trends and fluctuations and underlying mechanisms in terrestrial orchid populations.

Kobayashi, S., K. Inoue & M. Kato. 1997. Evidence of pollen transfer efficiency as the natural selection factor favoring a large corolla of *Campanula punctata* pollinated by *Bombus diversus*. Oecologia 111: 535-542.

Maad, J. 2000. Phenotypic selection in hawkmoth-pollinated *Platanthera bifolia*: Targets and fitness surfaces. Evolution 54: 112-123.

Morales Vargas, M. 2003. Variation and reproductive success across light environments in the common epiphytic orchid, *Tolumnia variegata* (Orchidaceae). M.S. Thesis, University of Puerto Rico, Río Piedras.

Montalvo, A.M. & J.D. Ackerman. 1987. Limitations to fruit production in *Ionopsis utricularioides*. Biotropica 19: 24-31.

Murren, C.J. & A.M. Ellison. 1996. Effects of habitat, plant size, and floral display on male and female reproductive success of the neotropical orchid *Brassavola nodosa*. Biotropica 28: 30-41.

Pedersen, H. A., S. Watthana, S. Suddee & S. Sasirat. 2004. Nat. Hit. Bull. Siam Soc. 52: 9-26.

Pfeifer, M., W. Heinrich & G. Jetschke. 2006. Climate, size and flowering history determine flowering pattern of *Himantoglossum hircinum*. Bot. J. Linnean Soc. 151:511-526.

Piper, J. G. & S. Waite. 1988. The gender role of flowers of broad leaved *Epipactis helleborine* (L.) Crantz (Orchidaceae). Funct. Ecol. 2:35-40.

Rossi, W., B. Corrias, P. Arduino, R. Cianchi & L. Bullini. 1992. Gene variation and gene flow in *Orchis morio* (Orchidaceae) from Italy. Pl. Syst. Evol 179: 43-58.

Sabat, A. M. & J. D. Ackerman 1996. Fruit set in a deceptive orchid: The effect of flowering phenology, display size, and local floral abundance. Amer. J. Bot. 83: 1181-1186.

Schemske, D.W. 1980. Evolution of floral display in the orchid *Brassavola nodosa*. Evolution 34: 489-493.

Smithson, A. & M. R. Macnair. 1997. Negative frequency-dependent selection by pollinators on artificial flowers without rewards. Evolution 51: 715-723.

Totland, O., H.L. Andersen, T. Bjelland, V. Dahl, W. Eide, S. Houge, T.R. Pedersen & E.U. Vie. 1998. Variation in pollen limitation among plants and phenotypic selection on floral traits in an early-spring flowering herb. Oikos 82: 491-501.

Tremblay, R. L. 2005. Larger is better: The effect of floral display on reproductive success in two populations of *Caladenia (Stegostyla) gracilis* R. Br. Muelleria 22: 77-85.

Tremblay, R. L,. J. D. Ackerman, J. K. Zimmerman & R. Calvo. 2005. Variation in Sexual reproduction in orchids and its evolutionary consequences: A spasmodic journey to diversification. Biol. J. Linnean Soc. 84: 1-54.

Tremblay, R. L., R. M. Bateman, A. Brown, M. Hachadourian, M. J. Hutchings, S. Kell, H. Koopowitz, C. Lehnebach & D. Whigham· Submitted. Density induced rates of pollinaria removal and deposition in the Purple Enamel-Orchid (*Elythranthera brunonis*). Lankesteriana, this issue.

Vallius, E. 2000. Position-dependent reproductive success of flowers in *Dactylorhiza maculata* (Orchidaceae). Func. Ecol. 14: 573-579.

Williams, J.L. & J.K. Conner. 2001. Sources of phenotypic variation in floral traits in wild radish, *Raphanus raphanistrum* (Brassicaceae). Amer. J. Bot. 88: 1577-1581.

Worley, A.C., A.M. Baker, J.D. Thompson & S.C.H. Barrett. 2000. Floral display in *Narcissus*: Variation in flower size and number at the species, population, and individual levels. Intern. J. Pl. Sc. 161: 69-79.

Zimmerman, J.K. & T.M. Aide. 1989. Patterns of fruit production in a neotropical orchid: pollinator vs. resource limitation. Amer. J. Bot. 76: 67-73.

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DENSITY INDUCED RATES OF POLLINARIA REMOVAL AND DEPOSITION IN THE PURPLE ENAMEL-ORCHID, *ELYTHRANTHERA BRUNONIS* (ENDL.) A.S. GEORGE

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RESUMEN. La distribución y densidad de los individuos dentro de las poblaciones de plantas pueden afectar el éxito reproductivo de sus integrantes. Luego de describir la filogenia de las orquideas del grupo de las Caladeniideas y su biología reproductiva, evaluamos el efecto de la densidad en el éxito reproductivo de la orquídea terrestre *Elythranthera brunonis*, endémica de Australia del Oeste. El éxito reproductivo de esta orquídea, medido como la deposición y remoción de polinios, fue evaluado. Se consideró como baja densidad aquellos individuos en los cuales se observó solamente una planta con flor en un radio de 2.5 m alrededor de esta planta focal y como alta densidad aquellas con una planta o más en un radio de 2.5 m alrededor de la planta focal. El éxito reproductivo de las plantas focales no fue afectado por la densidad de individuos en ninguna de las 6 poblaciones estudiadas. Sin embargo, cuando se evaluó la suma de las poblaciones en un conjunto, se observó que las plantas focales en "grupos" de mayor densidad tienen una mayor probabilidad de recibir polen. El número de flores por inflorescencia no afectó el éxito reproductivo de los individuos. Al contrario, el numero de flores total en un area de 2.5 m tenía un mayor éxito reproductivo en el componente de polinios removidos y depositados.

Introduction

Patterns of spatial distribution relative to conspecifics can affect the reproductive success of individual plants in a population if pollinators respond to floral display. Floral display can be perceived at one or more spatial scales by pollinators: the individual flowers, the number of open flowers on an inflorescence, the number of inflorescences on a plant, the number of plants in a definable cluster or in a definable population. The relationship between reproductive success and floral display has been studied in relatively few orchid species and no consistent pattern has yet emerged. For example, fruit production was greatest at intermediate inflorescence densities in *Calopogon tuberosus* (Firmage and Cole 1988), greatest at the highest inflorescence densities in *Anacamptis* (formerly *Orchis*) *morio* (Jersáková *et al.* 2002), and no relationship was detected between inflorescence density and fruit production in *Brassavola nodosa* (Schemske 1980), *Leporella fimbriata* (Peakall 1989), *Orchis purpurea* (Jacquemyn *et al.* 2002) or *Neottia* (formerly *Listera*) *cordata* (Meléndez-Ackerman & Ackerman 2001).

Data on the relationship between number of flowers in an inflorescence and fruit set in orchids, while scarce, are also inconsistent. In *Lepanthes wendlandii* (Calvo 1990), *Calopogon tuberosus* (Firmage & Cole 1988), *Ionopsis utricularioides* (Montalvo &

Ackerman 1987), *Orchis purpurea* (Jacquemyn *et al.* 2002) and *Aspasia principissa* (Zimmerman & Aide 1989), inflorescences bearing more flowers had a higher probability of setting fruit. In contrast, there was no effect of flower number per inflorescence on fruit set in *Psychilis krugii* (Ackerman 1989), *Epidendrum exasperatum* (Calvo 1990) or *Neotinea* (formerly *Orchis*) *ustulata* (Tali 1996). Inflorescence size may also affect male and female reproductive success. In *Epipactis helleborine*, Piper and Waite (1988) showed that the percentage of pollinaria exported and imported increased in a parallel fashion as inflorescence size increased, but the intercepts of the relationships were significantly different. Pollinaria export was significantly greater than pollinaria import at a given inflorescence size.

In this study we investigated two hypotheses related to reproductive success in the deceit-pollinated Purple Enamel-orchid, *Elythranthera brunonis* (Endl.) A.S. George, a terrestrial species endemic to Western Australia. The first hypothesis was that the reproductive success as measured from pollinaria deposition and removal of individual plants is independent of the local density of conspecific plants. As in neighborhood models of competition between plants (Pacala & Silander 1985, Jacquemyn *et al*. 2002), if resources – in this case pollinators – are limited, we would expect reproductive success to be lower in a higher density neighborhood if plants are deceit-pollinated and the animal vector(s) have the capacity to learn rapidly from their mistakes. The null hypothesis would be that more flowering plants per unit area could attract more pollinators, resulting in greater reproductive success per individual in denser populations. The second hypothesis was that both pollen removal (an index of male function) and pollen deposition (an index of female function) would be positively related to inflorescence size. In addition to presenting quantitative tests of these two hypotheses, we review the controversial taxonomic, phylogenetic and distributional contexts of our chosen study species, *Elythranthera brunonis*, which have not previously been collated in the literature.

Morphology and pollination biology

PHYLOGENETIC CONTEXT OF POLLINATION.

A molecular phylogenetic analysis combining the plastid regions *matK* and *trnL–F* (Kores *et al*. 2001, Hopper & Brown 2004) nested *Elythranthera* well within the "core Caladeniinae", one of three major clades that together constitute the re-circumscribed tribe Diurideae *s.s.* Recent morphological taxonomic studies have progressively disaggregated the exceptionally heterogeneous genus *Caladenia s.l.*, so that the "core Caladeniinae" now encompasses ten monophyletic genera, most of them containing few species (cf. Jones 1988, Hoffman & Brown 1998, Hopper & Brown 2001b). Four genera successively branch from the base of the clade Caladeniinae. *Adenochilus* (2 species, Eastern Australian/New Zealand) is succeeded by *Eriochilus* (8 species, mostly Western Australian), then *Leptoceras* (1 species, Western and Eastern Australian), then *Praecoxanthus* (1 species, Western Australian). A dichotomy then separates *Caladenia s.s.* (an estimated 243 mainly Australian species and 19 named hybrids in 6 subgenera) from a five-genus clade consisting of *Cyanicula* (10 species, both Western and Eastern Australian), *Pheladenia* (1 species, both Western and Eastern Australian), *Ericksonella* (1 species, Western Australian), and then the generic pairing of *Glossodia* (2 species, Eastern Australian) and *Elythranthera* (2 species, Western Australian) (Hopper and Brown 2004).

Members of the "core Caladeniinae" have a recognizable morphological "gestalt". The small underground tuber generates a single, fleshy leaf at or near the base of the slender stem, which in most cases is strongly hirsute and bears few flowers. The flowers are large relative to most other terrestrial orchids. In many species, the three sepals and two lateral petals are large, rhombic in outline, spreading and brightly colored, suggesting that they are primary visual attractants for pollinating insects. Deviations from this plesiomorphic condition occur in (a) the near-basal *Eriochilus* and *Leptoceras*, where the dorsal sepal and lateral petals are substantially reduced relative to the lateral sepals, and (b) the highly derived spider-orchids of *Caladenia* subgenus *Calonema*, subgenus *Drakonorchis* and subgenus *Phlebochilus,* which have strongly elongate sepals and lateral petals (e.g. Hopper & Brown 2001b). In all ten genera, the spurless labellum is well differentiated from the other five perianth segments by being more three-dimensional and much smaller, often possessing a fimbriate margin and/or adaxial calli. Together with the unusually elongate gynostemium, the labellum forms a visual and tactile focus for pollinators. The large gynostemium bears the relatively small, paired acrotenic pollinaria that are characteristic of the diurids (e.g. Dressler 1993, Pridgeon *et al*. 2001).

Focusing on the *Cyanicula–Glossodia–Elythranthera* clade, largely unpublished molecular phylogenetic data for the nrDNA internal transcribed spacer region demonstrate substantial divergence between the three genera (P. J. Kores, 2001, unpublished data; see also the preliminary ITS tree of Kores *et al. in* Hopper & Brown 2001a), though the occurrence of rare natural hybrids between *Cyanicula* and *Elythranthera* (Hoffman & Brown 1998) suggests that these genera are not wholly reproductively isolated.

The limited and often anecdotal information currently available (cf. Jones 2001, Dafni & Bernhardt 1990, A. P. Brown, 2006, unpublished data) suggests that the *Cyanicula, Elythranthera, Glossodia, Praecoxanthus* and the less derived subgenera of *Caladenia s.s.* are pollinated by unrewarded bees or, in the case of a few *Caladenia* and *Cyanicula* species, by beetles (Kores *et al*. 2001, A.P. Brown, 2006, unpublished data). Hoverflies and flower wasps are also known to be occasional pollinators, but these insects are thought to be sporadic visitors that rarely transfer pollen. The main exceptions lie in the species-rich genus *Caladenia*, where many spider-orchids of the more derived subgenera *Calonema, Drakonorchis* and *Phlebochilus* experience pheromonally-induced pseudocopulation by thynnid wasps (Stoutamire 1974, 1975, 1981, 1983), although some species also attract bees, flies and/or beetles (Bower 2001a; A.P. Brown, 2006, unpublished data). *Glossodia* species are pollinated by small bees of the genus *Halictus* (Jones 1988), as are both species of *Elythranthera*, which contain yellow-tipped calli that resemble anthers and hence may lure bees in search of pollen (A.P. Brown, 2006, unpublished data).

Thus, the core Caladeniinae encompass a wide range of floral morphologies that reflect an equal diversity of insect pollinators. Within this context, *Elythranthera* epitomises those genera that are highly attractive to bees but appear to offer them no tangible reward.

MORPHOLOGY AND AUTECOLOGY OF *ELYTHRANTHERA*.

Elythranthera was represented only by *E. emarginata* in the plastid phylogeny of Kores *et al.* (2001), but the second species in the genus, *E. brunonis*, has also been included in the forthcoming ITS phylogeny. This reveals a disparity of only four bases between the two species (P. J. Kores unpublished data. 2001), all perceived as autopomorphies of *E. brunonis*, suggesting that one species diverged from the other relatively recently.

Both species are distributed throughout south-west Western Australia and have broadly similar floral morphologies. The labellum bears two prominent calli and is smaller than the over-arching gynostemium, which superficially resembles an additional perianth segment due to its exceptionally well-developed lateral wings (see Pridgeon *et al*. 2001, fig. 28.1, pl. 7). The striking sepals are fleshy exceptionally glossy and somewhat recurved toward the apices.

Of the two species, *E. brunonis* is the more common, blooms earlier in the spring (between August and early November), is typically taller and bears smaller, purple rather than pink, flowers and more strongly folded labella (Jones 1988, Hoffman & Brown 1984, 1992, 1998, Brown 1999). Both species experience enhanced flowering following wildfire the previous summer (Hoffman & Brown 1992, 1998, Brown 1999; Jones 2001). *Elythranthera brunonis* occurs most commonly on the lateritic soils of the jarrah (*Eucalyptus marginata*) forest between Perth and Albany, though it is also found in inland woodlands and shrublands, seasonally wet swamplands and in areas of coastal heath over a wide area between Kalbarri on the west coast and Israelite Bay on the south coast. Our study sites encompassed a considerable portion of this geographical and ecological spectrum.

In most communities where it is found, *Elythranthera brunonis* occurs as scattered individuals (more rarely in small groups) that are typically spaced from 20 cm to several metres apart. This suggests that plants do not commonly multiply by producing more than one new tuber each year, and hence that reproduction is primarily by seed. It also renders *E. brunonis* an especially interesting model for the study of the influence of plant density on reproductive success.

Materials and methods

DATA COLLECTION AND ANALYSIS.

Data on reproductive success were collected over three consecutive days in September and October 2001 from six populations of *Elythranthera brunonis* in south-western Australia. Each site was visited once during a field trip that immediately followed the 1st World Orchid Conservation Congress in Perth (Dixon *et al.* 2003). Each of the authors targeted plants by randomly walking through each study site until a plant with at least one open flower was encountered. Once target plants were located, we evaluated the condition of each flower and counted the number fully open, partially open and in the bud stage. For fully open flowers, we determined whether at least

one of the two pollinaria had been removed from the gynostemium and whether or not pollen had been deposited on the stigma. We also counted the number of other individuals of *E. brunonis* flowering within a radius of 2.5 m of the target plant. Site locations and details of plant communities at each site are given in Table 1.

The effect on the reproductive success of target plants of being isolated (defined as being the only flowering plant of *Elythranthera brunonis* within 2.5 m or more clumped (defined as having at least one other flowering plant of *E. brunonis* within 2.5 m was tested by using a 2*2 contingency test by randomization. Because some expected frequencies were below 5, the computer program Fish6 Exact Test was used for analyses (Bill Engels, University of Wisconsin, 1992) rather than the Chi square test. To test whether there was a significant relationship between reproductive success and number of plants in a clump, a linear regression analysis was performed with all data combined, including isolated plants. Reproductive success is measured as the number of flowers with pollinaria removed from the bursicles and/or deposited on stigma divided by the total number of fully open flowers.

Results

Pollen removal and deposition.

Across all study sites, and irrespective of the number of neighbors around target plants, 10.1% of the flowers had clear evidence of pollinaria deposition and 15.9% of the flowers had clear evidence of at least one pollinarium removed.

Effect of inflorescence size.

Most plants had only one open flower (84.3%; 15.2% and 0.5% two and three flowered, respectively). The number of pollinaria removed and pollinaria deposited on stigmas did not differ significantly between plants that had two or more open flowers on the inflorescence, as compared with plants possessing only one open flower (Fisher's Exact test, pollinaria removal $p = 0.61$; pollinaria deposition $p = 0.14$: Table 3).

Plant isolation and reproductive success.

The probability of pollinaria being deposited and the probability of pollinaria being removed were not significantly affected by plants being isolated or in clumps when data from each of the six study sites were analyzed separately. However, when data from all sites were combined, the probability of pollinaria being deposited was significantly lower for isolated plants than for plants in clumps (Table 2).

Density-dependent reproductive success at the flower level.

A significant positive correlation ($r^2 = 22.5$) was observed between the percentage of flowers with pollinaria removal and the number of flowers within 2.5 m of the target plant (Kendall Rank Correlation, Tau corrected for ties 0.225, $p = 0.0001$, $n = 136$). A less strong, but still significant, positive correlation (18.5%) was observed for the percentage of flowers with pollinaria deposited and for number of flowers within 2.5 m of the target plant (Kendall Rank Correlation, Tau corrected for ties 0.185, $p = 0.001$, $n = 136$).

These results could be caused by the high proportion of all the recorded plants that were isolated. Thus, the analyses were repeated without the isolated individuals. The pattern remained the same for the effect of number of plants in the clump on pollinaria removal (Kendall Rank Correlation, Tau corrected for ties 0.268, $p = 0.006$, $n = 50$). However, the relationship between number of neighbors in the clump and the percentage of flowers on which pollinaria had been deposited became insignificant (Kendall Rank Correlation, Tau corrected for ties 0.138, $p = 0.16$, $n = 50$).

Discussion

Is *Elythranthera* truly deceptive?

Fruit production has generally been shown to be low in deceptive orchid species but, based on the literature (Tremblay *et al.* 2005), one cannot predict whether there will be a positive (e.g. this study; Jacquemyn *et al*. 2002a), negative (e.g. Fritz & Nilsson 1995) or neutral (e.g. Fritz & Nilsson 1995, Alexanderson & Ågren 1996, Tali 1996) relationship between inflorescence density and reproductive success. The range of responses observed thus far might be due to biological (e.g. pollinator availability, effects of herbivory, reproduction in previous years) and/or environmental controls (e.g. temporal variation in environmental quality and habitat disturbance). However, there is some evidence that reproductive success is temporally variable and that observations need to be made over long periods of time in order to fully understand the complex relationships between pollinators, the environment and the orchids.

Table 1. Details of sites where *Elythranthera brunonis* was sampled in Western Australia

Date	Site no.	Location	Latitude and Longitude	Habitat and associated orchids
30/09/01	1	Yallingup	33° 39' 30" S 115° 02' 20" E	Woodland of *Eucalyptus marginata, E. calophylla, Agonis flexuosa, Banksia attenuata* and *B. grandis* over shrubs of *Hibbertia hypericoides*, *Xanthorrhoea preisii, Acacia preisii* and *Orthrosanthus laxus*. Deep calcareous sand. Associated orchids included *Caladenia attingens, C. excelsa, C. flava, C. thinicola, Diuris* aff. *corymbosa, Lyperanthus serratus* and *Pterostylis recurva.*
30/09/01	2	Caves Road south-east of Redgate	34° 04' 03" S 115° 02' 10" E	Woodland of *Banksia attenuata, Eucalyptus marginata* and *Agonis flexuosa* over *Lysinema ciliatum, Adenanthos obovatus, Hibbertia hypericoides* and native sedges (*Lepidosperma* sp.). Deep grey sandy soil. Associated orchids included *Drakaea glyptodon, Paracaleana nigrita, Caladenia flava* and *Pterostylis turfosa.*
01/10/01	3	Lake Muir on the Muir Highway	34° 27' 03" S 116° 39' 01" E	Woodland of *Eucalyptus patens*, *Banksia littoralis* over *Melaleuca lanceolata, Hibbertia cuneata* and native sedges. Grey sandy rises above winter-wet flats. Associated orchids included *Caladenia flava, Lyperanthus serratus, Microtis media* and *Leptoceras menziesii.*
1/10/01	4	Two Peoples Bay, east of Albany	34° 57' 01" S 118° 11' 30" E	Open woodland of *Allocasuarina fraseri*, *Eucalyptus marginata*, *Banksia ilicifolia* over regenerating shrubs of *Johnsonia lupulina*, *Cosmelia rubra, Synaphea polymorpha* and *Xanthosia rotundifolia.* Deep grey sandy soil. Associated orchids included *Caladenia flava, Paracaleana nigrita* and *Prasophyllum elatum*. Burnt in January 2001.
02/10/01	5	Stirling Range National Park	34° 28' 20" S 118° 04' 05" E	Winter damp low shrubland of *Burtonia scabra, Andersonia simplex, Isopogon teretifolia, Lambertia inermis, Boronia inornata* and *Melaleuca suberosa*. Grey clay soil. Associated orchids included *Thelymitra villosa, T. crinita, T. flexuosa* and *Caladenia heberleana*.
04/10/01	6	Kings Park, Perth	31° 28' 22" S 115° 50' 10" E	Woodland of *Eucalyptus marginata*, *Banksia menziesii*, *B. attenuata*, *Allocasuarina fraseri* over *Hibbertia hypericoides, H. subvaginata.* Deep grey sandy soil. Associated orchids included *Caladenia flava, C. arenicola, C. discoidea, Pterostylis recurva*, *Thelymitra crinita* and *Pyrorchis nigricans*.

Table 2 The recorded number of flowers on isolated plants and plants in clumps, with and without evidence of pollinaria removal [PR] and pollinaria deposition [PD] on the stigma. P values indicate the results of Fisher's Exact tests to determine the probability that the distribution of the data departs from random expectation. N/A = data insufficient for valid statistical analysis.

	Number of flowering plants recorded	No. of plants without pollinaria removed	No. of plants with pollinaria removed	No. of plants without pollinaria depostited	No. of plants with pollinaria depostited	PR %	PD %
Site 1							
Isolated plants	12	12	0	11	1	0	6.7
Plants in clumps	27	27	0	27	0	0	0
P						N/A	0.129
Site 2							
Isolated plants	3	2	1	3	0	33.0	0
Plants in clumps	2	2	0	2	0	0	0
P						0.361	N/A
Site 3							
Isolated plants	23	20	3	22	1	13.0	4.3
Plants in clumps	24	21	3	23	1	12.5	4.2
P						0.908	0.975
Site 4							
Isolated plants	14	9	5	14	0	3.6	0
Plants in clumps	146	12	34	121	5	23.3	17.1
P						0.301	0.295
Site 5							
Isolated plants	31	31	0	31	0	0	0
Plants in clumps	8	8	0	8	0	0	0
P						N/A	N/A
Site 6							
Isolated plants	0	0	0	0	0	0	0
Plants in clumps	11	8	3	7	4	27.3	36.4
P						N/A	N/A
All data combined							
Isolated plants	83	74	9	81	2	10.8	.4
Plants in clumps	218	178	40	188	30	8.3	13.8
P						0.115	0.004

Table 3 The recorded number of plants with one or more than one flowers open, with and without evidence of pollinaria removal [PR] and pollinaria deposition [PD] on the stigma. P values indicate the results of Fisher's Exact tests to determine the probability that the distribution of the data departs from random expectation.

	Number of flowering plants recorded	No. of plants without pollinaria removed	No. of plants with pollinaria removed	No. of plants without pollinaria depostited	No. of plants with pollinaria depostited	PR %	PD %
Site 1							
Single Flower	253	213	40	229	24	15.8	9.5
Multi-Flowers	48	39	9	40	3	18.8	16.7
P						0.613	0.139

During a multi-year study, Primack and Stacey (1988) consistently found very low levels of reproductive success in *Cypripedium acaule*, a deceptive slipper orchid. Following major disturbances such as fire and defoliation of trees during a herbivorous insect outbreak, reproductive success was very high but only for a short period of time, and was positively correlated with a large increase in the number of flowering individuals following disturbance. This observation suggests that unpredictable and infrequent events (notably fire in the context of *Elythranthera brunonis*) may play a major role in determining long-term reproductive success in such species.

Like *Cypripedium acaule*, *Elythranthera brunonis* flowers in much larger numbers following summer wildfires (Hoffman & Brown 1992, 1998), clearly demonstrating that long-term monitoring and both long- and short-term manipulation studies such, as those performed on *Cypripedium acaule* (Gill 1996, Primack & Stacy 1998), *Neotinea ustulata* (Tali 2004), *Orchis simia* (Willems 2002) and *Dactylorhiza fuchsii* (Jersaková & Kindlmann 2002b), are needed to more fully understand the patterns and causes of temporal variation in orchid flowering and reproductive success.

Reproductive success is positively related to inflorescence size in some deceptive orchids (Jacquemyn *et al*. 2002a), although Calvo (1990) has suggested that it may not be an important factor in many species because of the low levels of pollination inherent in most orchids relative to comparable members of other plant families. Inflorescence size is probably of little consequence for reproductive success in *Elythranthera brunonis* because of the low variation in number of flowers per inflorescence. However, a study of the potential for selection on inflorescence size in *Stegostyla (*previously *Caladenia) gracilis* suggest that, if inflorescence size is genetically inherited and not environmentally determined, the potential for selection is present even if little variation in inflorescence size is observed in the field (Tremblay 2005). Higher reproductive success in clumps and larger floral densities suggests that, if the flowers of *E. brunonis* are indeed deceptive, the pollinators are slow to learn from their rewardless visits, or that larger groups of flowers are more attractive to potential pollinators. Because individual plants produce few flowers, the reproductive success of this species is mainly influenced by differences in inflorescence density. It would be interesting to know whether *E. brunonis* is indeed rewardless, and whether physical or chemical pollinator cues are present but have not yet been confidently identified.

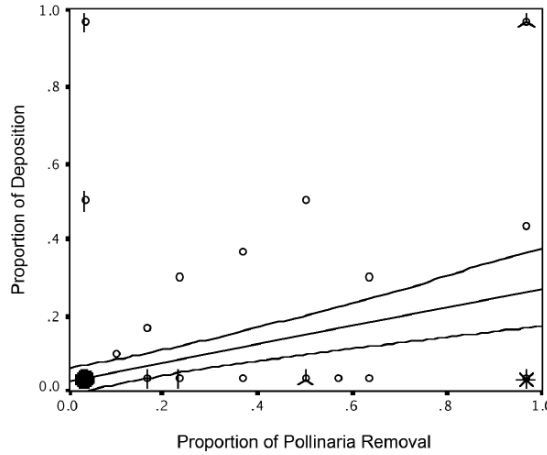

Figure 1 Pollen flux diagram for *Elythranthera brunonis*. For each individual the proportions of pollinated flowers (pollinaria deposition) is plotted against the proportions of flowers that have experience pollinaria removal. If pollinaria deposition and pollinaria removal occurred simultaneously then the points should lie on the diagonal. However, most of the points lie below this line, indicating that removals occurred prior to pollinaria deposition. The densities of the symbols (bars within a circle) represent larger sample size.

REPRODUCTIVE SUCCESS AND POLLINATOR LIMITATION.

As many studies have shown that orchids are pollinator limited (for an extensive review see Tremblay *et al.* 2005) we predicted that pollination would be lower in isolated plants. We found that fewer pollinaria were removed from flowers of isolated plants than from plants with close neighbors (0.11 vs 0.18), and that the effect of flower isolation on pollinaria deposition was equally significant (0.02 vs 0.14). These results indicate differences in the effect of local inflorescence density on male and female functional success.

The ratio of pollinaria removed versus pollinaria deposited was approximately 1.5:1 in *E. brunonis*. This ratio is high relative to that reported for several other orchids (Tremblay *et al*. 2005). However, a similar ratio was observed in the Puerto Rican species *Ionopsis utricularioides* = 1.96:1 (Montalvo & Ackerman 1987), *Comparettia falcata* = 1.35:1

(Salguero-Faría & Ackerman 1999), *Satyrium bicorne* = 0.30:1 (Ellis & Johnson 1999) and *Aerangis verdickii* = 2:1-3:1 (Light *et al*. 2004). All of these species offer nectar rewards which are much higher deposition to removal ratio as compared to Central American species *Stelis argentata* and the Brazilian species *Bulbophyllum ipanemense*, where the ratios are, respectively, 26.7:1 (Christensen 1992) and 24.2:1 (Borba & Semir 1998). Once again, the observed visitation rate of 10–15% suggests that *E. brunonis* is not a deceptive flower or at least offers a higher attracting ability then most deceptive species.

Our results suggest that there is a high efficiency, averaged across all sites, of transfer of pollen to the stigmatic surfaces. Assuming that removal and deposition of pollinaria were recorded with equal accuracy, our data suggest that approximately 64% of all pollinaria removed were eventually deposited on conspecific stigmas (i.e. there would have been a ratio of three pollinaria removed for every two deposited on a stigmatic surface). At present we are ignorant of the identity of the pollinator, (other than it is a bee in the genus *Halictus)*, and of the mechanism by which the insects are apparently attracted. The ratio of pollinaria deposited to pollinaria removed suggests that the pollination mechanism is effective on those occasions when visitation occurs. Knowledge of the species pollinating *E. brunonis* might help to explain the high efficiency of pollination.

Since none of the populations behaved significantly differently from each other there is no evidence to suggest that the effect of density on reproductive success is population/site dependent. Moreover, wherever this orchid is present it appears to be a common component of the flora regardless of the extent of its bushland habitat (i.e. small fragmented areas of remnant habitat or of larger areas of intact habitat: A.P. Brown, 2006, unpublished data). The observed variation in population density at the sampled sites may not necessarily represent the range of densities in this species, since fire can affect flowering densities and the fire histories of particular sampled sites are unknown.

If the pattern of reproductive success observed in *Elythranthera brunonis* during this short survey is consistent over longer periods then *E. brunonis* is partially affected by the spatial organization of individuals. Clumps of individuals have higher reproductive success through both male and female measures. Local pollination dynamics may be affected by other factors that mask the pollinator behavior in relation to the flowering densities. Furthermore, the high efficiency of pollinations suggests that *E. brunonis* is not a deceptive species and the autecology's of this species should to be investigated in greater depth.

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LITERATURE CITED

Ackerman, J.D. 1989. Limitations to sexual reproduction in *Encyclia krugii* (Orchidaceae). Syst. Bot. 14: 101-109.

Ackerman, J.D. 1990. Inflorescence size and fruit distribution among individuals of three orchid species. Amer. J. Bot. 77: 1378-1381.

Alexandersson ,R. & J. Ågren. 1996. Population size, pollinator visitation and fruit production in the deceptive orchid *Calypso bulbosa*. Oecologia 107: 533-540.

Borba E.L. & S. Semir. 1998. Wind-assisted fly pollination in three *Bulbophyllum* (Orchidaceae) species occurring in Brazilian campos rupestres. Lindleyana 13: 203-218.

Bower, C.C. 2001a. *Caladenia*: pollination. *In*: A.M. Pridgeon *et al*. (eds.). Genera Orchidacearum, 2; Orchidoideae 1. Oxford University Press. pp. 93-97.

Bower, C.C. 2001b. *Elythranthera*: pollination. *In*: A.M. Pridgeon *et al*. (eds.). Genera Orchidacearum, 2; Orchidoideae 1. Oxford University Press. pp. 103-104.

Brown, A.P. 1999. *Orchids of the South-West*. Department of Conservation and Land Management, Perth, Western Australia.

Calvo, R.N. 1990. Inflorescence size and fruit distribution among individuals of three orchid species. Amer. J. Bot. **77**: 1378-1381.

Christensen, D.E. 1992. Notes on the reproductive biology of *Stelis argentata* Lindl. (Orchidaceae: Pleurothallidinae) in eastern Ecuador. Lindleyana **7**: 28-33.

Dafni, A. & P. Bernhardt. 1990. Pollination of terrestrial orchids of southern Australia and the Mediterannean region: systematic, ecological and evolutionary implications. Evol. Biol. 24: 193-253.

Dixon, K.W., S.P. Kell, R.L. Barrett & P.J. Cribb (eds.). 2003. Orchid Conservation. Natural History Publications (Borneo), Kota Kinabalu, Sabah, Malaysia.

Dressler, R.L. 1993. Phylogeny and Classification of the Orchid Family. Cambridge University Press, Cambridge.

Ellis, A.G. & S.D. Johnson. 1999. Do pollinators deter-

mine hybridization patterns in sympatric *Satyrium* (Orchidaceae) species? Pl. Syste. Evol. 219: 137-150.

Firmage, D.H. & F.R. Cole. 1988. Reproductive success and inflorescence size of *Calopogon tuberosus* (Orchidaceae). Amer. J. Bot. 75: 1371-1377.

Fritz, A.L. & L.A. Nilsson. 1994.How pollinator-mediated mating varies with population size in plants. Oecologia 100: 451-462.

George, A.S. 1963. Notes on western Australian Orchidaceae. III. *Elythranthera*, a new genus for the enamel orchids. Western Australia Naturalist 9: 2-9.

Gill, D.E. 1996. The natural population ecology of temperate terrestrials: Pink lady's slippers, *Cypripedium acaule*. *In*: C. Allen (ed.). North American Native Terrestrial Orchids: Propagation and Production. North American Native Terrestrial Orchid Conference, Germantown, MD. pp 91-106.

Hoffman, N. & A. Brown. 1984. Orchids of South-West Australia. First Edition. University of Western Australia Press, Nedlands.

Hoffman, N. & A. Brown. 1992. Orchids of South-West Australia. Second Edition. University of Western Australia Press: Nedlands.

Hoffman, N. & A. Brown. 1998. Orchids of South-West Australia. Revised Second Edition with supplement. University of Western Australia Press: Nedlands.

Hopper, S.D. & A.P. Brown. 2001a. Contributions to Western Australian orchidology: 2. New taxa and circumscriptions in *Caladenia* (Spider, Fairy and Dragon Orchids of Western Australia). Nuytsia 14: 27-314.

Hopper, S.D. & A.P. Brown. 2001b. Contributions to Western Australian orchidology: 1. History of early collections, taxonomic concepts and key to genera. Nuytsia 14: 1-26.

Jacquemyn, H., R. Brys & M. Hermy. 2002. Flower and fruit production in small populations of *Orchis purpurea* and implications for management. *In*: P. Kindlmann, J.H. Willems & D.H. Whigham (eds). Trends and Fluctuations and Underlying Mechanisms in Terrestrial Orchid Populations. Backhuys Publishers, Leiden, the Netherlands. pp. 67-84.

Jersákova J., P. Kindlmann & M. Stritesky. 2002. Population dynamics of *Orchis morio* in the Czech Republic under human influence. *In*: P. Kindlmann, J.H. Willems & D.H. Whigham (eds). Trends and Fluctuations and Underlying Mechanisms in Terrestrial Orchid Populations. Backhuys Publishers, Leiden, the Netherlands. pp. 187-208.

Jersákova, J. & P. Kindlmann. 2002. Key factors affecting shoot growth and flowering performance of *Dactylorhiza fuchsii*. *In*: P. Kindlmann, J.H. Willems & D.H. Whigham (eds). Trends and Fluctuations and Underlying Mechanisms in Terrestrial Orchid Populations. Backhuys Publishers, Leiden, the Netherlands. pp. 99-113.

Jones, D.L. 1988. Native Orchids of Australia. Reed Books, Frenchs Forest, Australia.

Jones, D.L. 2001. Subtribe Caladeniinae. *In*: A.M. Pridgeon *et al.* (eds.). Genera Orchidacearum, 2; Orchidoideae 1. Oxford University Press. pp. 83-115.

Kores P.J., M. Molvray, P.H. Weston, S.D. Hopper, A.P. Brown A. P., K.M. Cameron & M.W. Chase. 2001. A phylogenetic analysis of Diurideae (Orchidaceae) based on plastid DNA sequence data. Amer. J. Bot. 88: 1903–1914.

Light M., H. Koopowitz & T. Marchant. 2003. The impact of climatic, edaphic and physiographic factors on the population behaviour of selected temperate and tropical orchids. *In*: K.W. Dixon, S.P. Kell, R.L. Barrett & P.J. Cribb (eds.). 2003. *Orchid Conservation*. Natural History Publications (Borneo), Kota Kinabalu, Sabah, Malaysia. pp. 357-382.

Meléndez-Ackerman, E.J. & J.D. Ackerman. 2001. Density-dependent variation in reproductive success in a terrestrial orchid. Pl. Syst. Evol. 227: 27-36.

Montalvo, A.M. & J.D. Ackerman. 1987. Limitations to fruit production in *Ionopsis utricularioides*. Biotropica 19: 24-31.

Pacala, S.W. & Jr. Silander. 1985. Neighborhood models of plant population dynamics I. Single–species models of annuals. Amer. Naturalist 125: 385-411.

Peakall, R. 1989. The unique pollination of *Leporella fimbriata* (Orchidaceae): pollination by pseudocopulating male ants (*Myrmeca urens*, Formicidae).Pl. Syst. Evol. 167: 137-148.

Piper, J.G. & S. Waite. 1988. The gender role of flowers of broad-leaved helleborine, *Epipactis helleborine* (L.) Crantz (Orchidaceae). Functional Ecology 2: 35-40.

Pridgeon, A.M., P.J. Cribb, M.W. Chase & F.N Rasmussen (eds). 2001. Genera Orchidacearum. 2. Orchidoideae, Part 1. Oxford University Press, Oxford.

Primack, R. & E. Stacy. 1988.Cost of reproduction in the pink lady's slipper (*Cypripedium acaule*, Orchidaceae): An eleven-year experimental study of three populations. Amer. J. Bot. 85: 1672-1679.

Sabat, A.M. & J.D. Ackerman. 1996. Fruit set in a deceptive orchid: the effect of flowering phenology, display size, and local floral abundance. Amer. J. Bot. 83: 1181-1186.

Salguero-Faría, J.A. & J.D. Ackerman. 1999. A nectar reward: is more better? Biotropica 31: 303-311.

Schemske, D.W. 1980. Evolution of floral display in the orchid *Brassavola nodosa*. Evolution 34: 489-493.

Snow, A.A. & D.F. Whigham. 1989. Cost of flower and fruit production in *Tipularia discolor*. Ecology 70: 1286-1293.

Stoutamire, W.P. 1974. Australian terrestrial orchids, thynnid wasps, and pseudocopulation. Amer. orch. Soc. Bull.

43: 13–18.
Stoutamire, W.P. 1975. Pseudocopulation in Australian terrestrial orchids.Amer. orch. Soc. Bull. 44: 226–233.
Stoutamire, W.P. 1981.Pollination studies in Australian terrestrial orchids. National Geographic Society Research Reports 13: 591–98.
Stoutamire, W.P. 1983. Wasp-pollinated species of *Caladenia* (Orchidaceae) in South-western Australia. Austral. J. Bot. 31: 383–394.
Tali, K. 1996. Spring-flowering and summer-flowering populations of *Orchis ustulata* L. (Orchidaceae) in Estonia; their comparison and distribution. J. Europ. Orch. 28: 573-582.
Tali, K. 2004. Species structure in *Neotinea ustulata*. PhD Thesis, University of Tartu, Tartu, Estonia.
Tremblay, R. L., J.D. Ackerman, J.K. Zimmerman & R. Calvo. 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. Biol. J. Linnean Soc. 84: 1-54.
Tremblay, R.L. 2005. Larger is better: The effect of floral display on reproductive success in two populations of *Caladenia* (*Stegostyla) gracilis* R. Br. Muelleria 22: 77-85.
Willem, J. 2002. A founder population of *Orchis simia* in the Netherlands. *In*: P. Kindlmann, J.H. Willems & D.H. Whigham (eds). Trends and Fluctuations and Underlying Mechanisms in Terrestrial Orchid Populations. Backhuys Publishers, Leiden, the Netherlands, pp. 23-33.
Zimmerman, J. K. & T.M. Aide. 1989. Patterns of fruit production in a neotropical orchid: pollinator vs. resource limitation. Amer. J. Bot. 76: 67-73.

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DENSITY INDUCED RATES OF POLLINARIA REMOVAL AND DEPOSITION IN THE PURPLE ENAMEL-ORCHID, *ELYTHRANTHERA BRUNONIS* (ENDL.) A.S. GEORGE

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RESUMEN. La distribución y densidad de los individuos dentro de las poblaciones de plantas pueden afectar el éxito reproductivo de sus integrantes. Luego de describir la filogenia de las orquideas del grupo de las Caladeniideas y su biología reproductiva, evaluamos el efecto de la densidad en el éxito reproductivo de la orquídea terrestre *Elythranthera brunonis*, endémica de Australia del Oeste. El éxito reproductivo de esta orquídea, medido como la deposición y remoción de polinios, fue evaluado en seis poblaciones de diferentes densidades. Se consideró como baja densidad aquellas poblaciones en las cuales se observó al menos una planta en flor en un radio de 2.5 m alrededor de una planta focal y como alta densidad a aquellas poblaciones con mas de una planta en un radio de 2.5 m alrededor de la planta focal. El éxito reproductivo de las plantas focales no fue afectado por la densidad de individuos en ninguna de las 6 poblaciones estudiadas. Sin embargo, cuando se evaluó la suma de las poblaciones en un conjunto, se observó que las plantas focales en poblaciones de mayor densidad tienen una mayor probabilidad de recibir polen. El número de flores por inflorescencia no afectó el éxito reproductivo de los individuos. Al contrario, el numero de flores total en un area de 2.5 m tenía un mayor éxito reproductivo en el componente de polinios removidos y depositados.

Introduction

Patterns of spatial distribution relative to conspecifics can affect the reproductive success of individual plants in a population if pollinators respond to floral display. Floral display can be perceived at one or more spatial scales by pollinators: the individual flowers, the number of open flowers on an inflorescence, the number of inflorescences on a plant, the number of plants in a definable cluster or in a definable population. The relationship between reproductive success and floral display has been studied in relatively few orchid species and no consistent pattern has yet emerged. For example, fruit production was greatest at intermediate inflorescence densities in *Calopogon tuberosus* (Firmage and Cole 1988), greatest at the highest inflorescence densities in *Anacamptis* (formerly *Orchis*) *morio* (Jersáková *et al.* 2002), and no relationship was detected between inflorescence density and fruit production in *Brassavola nodosa* (Schemske 1980), *Leporella fimbriata* (Peakall 1989), *Orchis purpurea* (Jacquemyn *et al*. 2002) or *Neottia* (formerly *Listera*) *cordata* (Meléndez-Ackerman & Ackerman 2001).

Data on the relationship between number of flowers in an inflorescence and fruit set in orchids, while scarce, are also inconsistent. In *Lepanthes wendlandii* (Calvo 1990), *Calopogon tuberosus* (Firmage & Cole 1988), *Ionopsis utricularioides* (Montalvo &

Ackerman 1987), *Orchis purpurea* (Jacquemyn *et al.* 2002) and *Aspasia principissa* (Zimmerman & Aide 1989), inflorescences bearing more flowers had a higher probability of setting fruit. In contrast, there was no effect of flower number per inflorescence on fruit set in *Psychilis krugii* (Ackerman 1989), *Epidendrum exasperatum* (Calvo 1990) or *Neotinea* (formerly *Orchis*) *ustulata* (Tali 1996). Inflorescence size may also affect male and female reproductive success. In *Epipactis helleborine*, Piper and Waite (1988) showed that the percentage of pollinaria exported and imported increased in a parallel fashion as inflorescence size increased, but the intercepts of the relationships were significantly different. Pollinaria export was significantly greater than pollinaria import at a given inflorescence size.

In this study we investigated two hypotheses related to reproductive success in the deceit-pollinated Purple Enamel-orchid, *Elythranthera brunonis* (Endl.) A.S. George, a terrestrial species endemic to Western Australia. The first hypothesis was that the reproductive success as measured from pollinaria deposition and removal of individual plants is independent of the local density of conspecific plants. As in neighborhood models of competition between plants (Pacala & Silander 1985, Jacquemyn *et al.* 2002), if resources – in this case pollinators – are limited, we would expect reproductive success to be lower in a higher density neighborhood if plants are deceit-pollinated and the animal vector(s) have the capacity to learn rapidly from their mistakes. The null hypothesis would be that more flowering plants per unit area could attract more pollinators, resulting in greater reproductive success per individual in denser populations. The second hypothesis was that both pollen removal (an index of male function) and pollen deposition (an index of female function) would be positively related to inflorescence size. In addition to presenting quantitative tests of these two hypotheses, we review the controversial taxonomic, phylogenetic and distributional contexts of our chosen study species, *Elythranthera brunonis*, which have not previously been collated in the literature.

Morphology and pollination biology

PHYLOGENETIC CONTEXT OF POLLINATION.

A molecular phylogenetic analysis combining the plastid regions *matK* and *trnL–F* (Kores *et al.* 2001, Hopper & Brown 2004) nested *Elythranthera* well within the "core Caladeniinae", one of three major clades that together constitute the re-circumscribed tribe Diurideae *s.s.* Recent morphological taxonomic studies have progressively disaggregated the exceptionally heterogeneous genus *Caladenia s.l.*, so that the "core Caladeniinae" now encompasses ten monophyletic genera, most of them containing few species (cf. Jones 1988, Hoffman & Brown 1998, Hopper & Brown 2001b). Four genera successively branch from the base of the clade Caladeniinae. *Adenochilus* (2 species, Eastern Australian/New Zealand) is succeeded by *Eriochilus* (8 species, mostly Western Australian), then *Leptoceras* (1 species, Western and Eastern Australian), then *Praecoxanthus* (1 species, Western Australian). A dichotomy then separates *Caladenia s.s.* (an estimated 243 mainly Australian species and 19 named hybrids in 6 subgenera) from a five-genus clade consisting of *Cyanicula* (10 species, both Western and Eastern Australian), *Pheladenia* (1 species, both Western and Eastern Australian), *Ericksonella* (1 species, Western Australian), and then the generic pairing of *Glossodia* (2 species, Eastern Australian) and *Elythranthera* (2 species, Western Australian) (Hopper and Brown 2004).

Members of the "core Caladeniinae" have a recognizable morphological "gestalt". The small underground tuber generates a single, fleshy leaf at or near the base of the slender stem, which in most cases is strongly hirsute and bears few flowers. The flowers are large relative to most other terrestrial orchids. In many species, the three sepals and two lateral petals are large, rhombic in outline, spreading and brightly colored, suggesting that they are primary visual attractants for pollinating insects. Deviations from this plesiomorphic condition occur in (a) the near-basal *Eriochilus* and *Leptoceras*, where the dorsal sepal and lateral petals are substantially reduced relative to the lateral sepals, and (b) the highly derived spider-orchids of *Caladenia* subgenus *Calonema*, subgenus *Drakonorchis* and subgenus *Phlebochilus,* which have strongly elongate sepals and lateral petals (e.g. Hopper & Brown 2001b). In all ten genera, the spurless labellum is well differentiated from the other five perianth segments by being more three-dimensional and much smaller, often possessing a fimbriate margin and/or adaxial calli. Together with the unusually elongate gynostemium, the labellum forms a visual and tactile focus for pollinators. The large gynostemium bears the relatively small, paired acrotenic pollinaria that are characteristic of the diurids (e.g. Dressler 1993, Pridgeon *et al.* 2001).

Focusing on the *Cyanicula–Glossodia–Elythranthera* clade, largely unpublished molecular phylogenetic data for the nrDNA internal transcribed spacer region demonstrate substantial divergence between the three genera (P. J. Kores, 2001, unpublished data; see also the preliminary ITS tree of Kores *et al. in* Hopper & Brown 2001a), though the occurrence of rare natural hybrids between *Cyanicula* and *Elythranthera* (Hoffman & Brown 1998) suggests that these genera are not wholly reproductively isolated.

The limited and often anecdotal information currently available (cf. Jones 2001, Dafni & Bernhardt 1990, A. P. Brown, 2006, unpublished data) suggests that the *Cyanicula, Elythranthera, Glossodia, Praecoxanthus* and the less derived subgenera of *Caladenia s.s.* are pollinated by unrewarded bees or, in the case of a few *Caladenia* and *Cyanicula* species, by beetles (Kores *et al*. 2001, A.P. Brown, 2006, unpublished data). Hoverflies and flower wasps are also known to be occasional pollinators, but these insects are thought to be sporadic visitors that rarely transfer pollen. The main exceptions lie in the species-rich genus *Caladenia*, where many spider-orchids of the more derived subgenera *Calonema, Drakonorchis* and *Phlebochilus* experience pheromonally-induced pseudocopulation by thynnid wasps (Stoutamire 1974, 1975, 1981, 1983), although some species also attract bees, flies and/or beetles (Bower 2001a; A.P. Brown, 2006, unpublished data). *Glossodia* species are pollinated by small bees of the genus *Halictus* (Jones 1988), as are both species of *Elythranthera*, which contain yellow-tipped calli that resemble anthers and hence may lure bees in search of pollen (A.P. Brown, 2006, unpublished data).

Thus, the core Caladeniinae encompass a wide range of floral morphologies that reflect an equal diversity of insect pollinators. Within this context, *Elythranthera* epitomises those genera that are highly attractive to bees but appear to offer them no tangible reward.

MORPHOLOGY AND AUTECOLOGY OF *ELYTHRANTHERA*.

Elythranthera was represented only by *E. emarginata* in the plastid phylogeny of Kores *et al.* (2001), but the second species in the genus, *E. brunonis*, has also been included in the forthcoming ITS phylogeny. This reveals a disparity of only four bases between the two species (P. J. Kores unpublished data. 2001), all perceived as autopomorphies of *E. brunonis*, suggesting that one species diverged from the other relatively recently.

Both species are distributed throughout south-west Western Australia and have broadly similar floral morphologies. The labellum bears two prominent calli and is smaller than the over-arching gynostemium, which superficially resembles an additional perianth segment due to its exceptionally well-developed lateral wings (see Pridgeon *et al*. 2001, fig. 28.1, pl. 7). The striking sepals are fleshy exceptionally glossy and somewhat recurved toward the apices.

Of the two species, *E. brunonis* is the more common, blooms earlier in the spring (between August and early November), is typically taller and bears smaller, purple rather than pink, flowers and more strongly folded labella (Jones 1988, Hoffman & Brown 1984, 1992, 1998, Brown 1999). Both species experience enhanced flowering following wildfire the previous summer (Hoffman & Brown 1992, 1998, Brown 1999; Jones 2001). *Elythranthera brunonis* occurs most commonly on the lateritic soils of the jarrah (*Eucalyptus marginata*) forest between Perth and Albany, though it is also found in inland woodlands and shrublands, seasonally wet swamplands and in areas of coastal heath over a wide area between Kalbarri on the west coast and Israelite Bay on the south coast. Our study sites encompassed a considerable portion of this geographical and ecological spectrum.

In most communities where it is found, *Elythranthera brunonis* occurs as scattered individuals (more rarely in small groups) that are typically spaced from 20 cm to several metres apart. This suggests that plants do not commonly multiply by producing more than one new tuber each year, and hence that reproduction is primarily by seed. It also renders *E. brunonis* an especially interesting model for the study of the influence of plant density on reproductive success.

Materials and methods

DATA COLLECTION AND ANALYSIS.

Data on reproductive success were collected over three consecutive days in September and October 2001 from six populations of *Elythranthera brunonis* in south-western Australia. each site was visited once during a field trip that immediately followed the 1st World Orchid Conservation Congress in Perth (Dixon *et al.* 2003). Each of the authors targeted plants by randomly walking through each study site until a plant with at least one open flower was encountered. Once target plants were located, we evaluated the condition of each flower and counted the number fully open, partially open and in the bud stage. For fully open flowers, we determined whether at least

one of the two pollinaria had been removed from the gynostemium and whether or not pollen had been deposited on the stigma. We also counted the number of other individuals of *E. brunonis* flowering within a radius of 2.5 m of the target plant. Site locations and details of plant communities at each site are given in Table 1.

The effect on the reproductive success of target plants of being isolated (defined as being the only flowering plant of *Elythranthera brunonis* within 2.5 m or more clumped (defined as having at least one other flowering plant of *E. brunonis* within 2.5 m was tested by using a 2*2 contingency test by randomization. Because some expected frequencies were below 5, the computer program Fish6 Exact Test was used for analyses (Bill Engels, University of Wisconsin, 1992) rather than the Chi square test. To test whether there was a significant relationship between reproductive success and number of plants in a clump, a linear regression analysis was performed with all data combined, including isolated plants. Reproductive success is measured as the number of flowers with pollinaria removed from the bursicles and/or deposited on stigma divided by the total number of fully open flowers.

Results

Pollen removal and deposition.

Across all study sites, and irrespective of the number of neighbors around target plants, 10.1% of the flowers had clear evidence of pollinaria deposition and 15.9% of the flowers had clear evidence of at least one pollinarium removed.

Effect of inflorescence size.

Most plants had only one open flower (84.3%; 15.2% and 0.5% two and three flowered, respectively). The number of pollinaria removed and pollinaria deposited on stigmas did not differ significantly between plants that had two or more open flowers on the inflorescence, as compared with plants possessing only one open flower (Fisher's Exact test, pollinaria removal $p = 0.61$; pollinaria deposition $p = 0.14$: Table 3).

Plant isolation and reproductive success.

The probability of pollinaria being deposited and the probability of pollinaria being removed were not significantly affected by plants being isolated or in clumps when data from each of the six study sites were analyzed separately. However, when data from all sites were combined, the probability of pollinaria being deposited was significantly lower for isolated plants than for plants in clumps (Table 2).

Density-dependent reproductive success at the flower level.

A significant positive correlation ($r^2 = 22.5$) was observed between the percentage of flowers with pollinaria removal and the number of flowers within 2.5 m of the target plant (Kendall Rank Correlation, Tau corrected for ties 0.225, $p = 0.0001$, $n = 136$). A less strong, but still significant, positive correlation (18.5%) was observed for the percentage of flowers with pollinaria deposited and for number of flowers within 2.5 m of the target plant (Kendall Rank Correlation, Tau corrected for ties 0.185, $p = 0.001$, $n = 136$).

These results could be caused by the high proportion of all the recorded plants that were isolated. Thus, the analyses were repeated without the isolated individuals. The pattern remained the same for the effect of number of plants in the clump on pollinaria removal (Kendall Rank Correlation, Tau corrected for ties 0.268, $p = 0.006$, $n = 50$). However, the relationship between number of neighbors in the clump and the percentage of flowers on which pollinaria had been deposited became insignificant (Kendall Rank Correlation, Tau corrected for ties 0.138, $p = 0.16$, $n = 50$).

Discussion

Is *Elythranthera* truly deceptive?

Fruit production has generally been shown to be low in deceptive orchid species but, based on the literature (Tremblay *et al.* 2005), one cannot predict whether there will be a positive (e.g. this study; Jacquemyn *et al*. 2002a), negative (e.g. Fritz & Nilsson 1995) or neutral (e.g. Fritz & Nilsson 1995, Alexanderson & Ågren 1996, Tali 1996) relationship between inflorescence density and reproductive success. The range of responses observed thus far might be due to biological (e.g. pollinator availability, effects of herbivory, reproduction in previous years) and/or environmental controls (e.g. temporal variation in environmental quality and habitat disturbance). However, there is some evidence that reproductive success is temporally variable and that observations need to be made over long periods of time in order to fully understand the complex relationships between pollinators, the environment and the orchids.

Table 1. Details of sites where *Elythranthera brunonis* was sampled in Western Australia

Date	Site no.	Location	Latitude and Longitude	Habitat and associated orchids
30/09/01	1	Yallingup	33° 39' 30" S 115° 02' 20" E	Woodland of *Eucalyptus marginata, E. calophylla, Agonis flexuosa, Banksia attenuata* and *B. grandis* over shrubs of *Hibbertia hypericoides*, *Xanthorrhoea preisii, Acacia preisii* and *Orthrosanthus laxus*. Deep calcareous sand. Associated orchids included *Caladenia attingens, C. excelsa, C. flava, C. thinicola, Diuris* aff. *corymbosa, Lyperanthus serratus* and *Pterostylis recurva.*
30/09/01	2	Caves Road south-east of Redgate	34° 04' 03" S 115° 02' 10" E	Woodland of *Banksia attenuata, Eucalyptus marginata* and *Agonis flexuosa* over *Lysinema ciliatum, Adenanthos obovatus, Hibbertia hypericoides* and native sedges (*Lepidosperma* sp.). Deep grey sandy soil. Associated orchids included *Drakaea glyptodon, Paracaleana nigrita, Caladenia flava* and *Pterostylis turfosa.*
01/10/01	3	Lake Muir on the Muir Highway	34° 27' 03" S 116° 39' 01" E	Woodland of *Eucalyptus patens*, *Banksia littoralis* over *Melaleuca lanceolata, Hibbertia cuneata* and native sedges. Grey sandy rises above winter-wet flats. Associated orchids included *Caladenia flava, Lyperanthus serratus, Microtis media* and *Leptoceras menziesii.*
1/10/01	4	Two Peoples Bay, east of Albany	34° 57' 01" S 118° 11' 30" E	Open woodland of *Allocasuarina fraseri*, *Eucalyptus marginata*, *Banksia ilicifolia* over regenerating shrubs of *Johnsonia lupulina*, *Cosmelia rubra, Synaphea polymorpha* and *Xanthosia rotundifolia.* Deep grey sandy soil. Associated orchids included *Caladenia flava, Paracaleana nigrita* and *Prasophyllum elatum*. Burnt in January 2001.
02/10/01	5	Stirling Range National Park	34° 28' 20" S 118° 04' 05" E	Winter damp low shrubland of *Burtonia scabra, Andersonia simplex, Isopogon teretifolia, Lambertia inermis, Boronia inornata* and *Melaleuca suberosa*. Grey clay soil. Associated orchids included *Thelymitra villosa, T. crinita, T. flexuosa* and *Caladenia heberleana*.
04/10/01	6	Kings Park, Perth	31° 28' 22" S 115° 50' 10" E	Woodland of *Eucalyptus marginata*, *Banksia menziesii*, *B. attenuata*, *Allocasuarina fraseri* over *Hibbertia hypericoides, H. subvaginata.* Deep grey sandy soil. Associated orchids included *Caladenia flava, C. arenicola, C. discoidea, Pterostylis recurva*, *Thelymitra crinita* and *Pyrorchis nigricans*.

Table 2 The recorded number of flowers on isolated plants and plants in clumps, with and without evidence of pollinaria removal [PR] and pollinaria deposition [PD] on the stigma. P values indicate the results of Fisher's Exact tests to determine the probability that the distribution of the data departs from random expectation. N/A = data insufficient for valid statistical analysis.

	Number of flowering plants recorded	No. of plants without pollinaria removed	No. of plants with pollinaria removed	No. of plants without pollinaria depostited	No. of plants with pollinaria depostited	PR %	PD %
Site 1							
Isolated plants	12	12	0	11	1	0	6.7
Plants in clumps	27	27	0	27	0	0	0
P						N/A	0.129
Site 2							
Isolated plants	3	2	1	3	0	33.0	0
Plants in clumps	2	2	0	2	0	0	0
P						0.361	N/A
Site 3							
Isolated plants	23	20	3	22	1	13.0	4.3
Plants in clumps	24	21	3	23	1	12.5	4.2
P						0.908	0.975
Site 4							
Isolated plants	14	9	5	14	0	3.6	0
Plants in clumps	146	12	34	121	5	23.3	17.1
P						0.301	0.295
Site 5							
Isolated plants	31	31	0	31	0	0	0
Plants in clumps	8	8	0	8	0	0	0
P						N/A	N/A
Site 6							
Isolated plants	0	0	0	0	0	0	0
Plants in clumps	11	8	3	7	4	27.3	36.4
P						N/A	N/A
All data combined							
Isolated plants	83	74	9	81	2	10.8	.4
Plants in clumps	218	178	40	188	30	8.3	13.8
P						0.115	0.004

Table 3 The recorded number of plants with one or more than one flowers open, with and without evidence of pollinaria removal [PR] and pollinaria deposition [PD] on the stigma. P values indicate the results of Fisher's Exact tests to determine the probability that the distribution of the data departs from random expectation. N/A = data insufficient for valid statistical analysis.

	Number of flowering plants recorded	No. of plants without pollinaria removed	No. of plants with pollinaria removed	No. of plants without pollinaria depostited	No. of plants with pollinaria depostited	PR %	PD %
Site 1							
Single Flowe	253	213	40	229	24	15.8	9.5
Multi-Flowers	48	39	9	40	3	18.8	16.7
P						0.613	0.139

During a multi-year study, Primack and Stacey (1988) consistently found very low levels of reproductive success in *Cypripedium acaule*, a deceptive slipper orchid. Following major disturbances such as fire and defoliation of trees during a herbivorous insect outbreak, reproductive success was very high but only for a short period of time, and was positively correlated with a large increase in the number of flowering individuals following disturbance. This observation suggests that unpredictable and infrequent events (notably fire in the context of *Elythranthera brunonis*) may play a major role in determining long-term reproductive success in such species.

Like *Cypripedium acaule*, *Elythranthera brunonis* flowers in much larger numbers following summer wildfires (Hoffman & Brown 1992, 1998), clearly demonstrating that long-term monitoring and both long- and short-term manipulation studies such, as those performed on *Cypripedium acaule* (Gill 1996, Primack & Stacy 1998), *Neotinea ustulata* (Tali 2004), *Orchis simia* (Willems 2002) and *Dactylorhiza fuchsii* (Jersaková & Kindlmann 2002b), are needed to more fully understand the patterns and causes of temporal variation in orchid flowering and reproductive success.

Reproductive success is positively related to inflorescence size in some deceptive orchids (Jacquemyn *et al*. 2002a), although Calvo (1990) has suggested that it may not be an important factor in many species because of the low levels of pollination inherent in most orchids relative to comparable members of other plant families. Inflorescence size is probably of little consequence for reproductive success in *Elythranthera brunonis* because of the low variation in number of flowers per inflorescence. However, a study of the potential for selection on inflorescence size in *Stegostyla (*previously *Caladenia) gracilis* suggest that, if inflorescence size is genetically inherited and not environmentally determined, the potential for selection is present even if little variation in inflorescence size is observed in the field (Tremblay 2005). Higher reproductive success in clumps and larger floral densities suggests that, if the flowers of *E. brunonis* are indeed deceptive, the pollinators are slow to learn from their rewardless visits, or that larger groups of flowers are more attractive to potential pollinators. Because individual plants produce few flowers, the reproductive success of this species is mainly influenced by differences in inflorescence density. It would be interesting to know whether *E. brunonis* is indeed rewardless, and whether physical or chemical pollinator cues are present but have not yet been confidently identified.

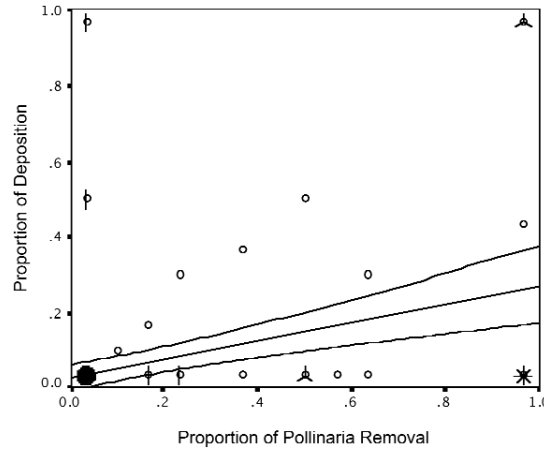

Figure 1 Pollen flux diagram for *Elythranthera brunonis*. For each individual the proportions of pollinated flowers (pollinaria deposition) is plotted against the proportions of flowers that have experience pollinaria removal. If pollinaria deposition and pollinaria removal occurred simultaneously then the points should lie on the diagonal. However, most of the points lie below this line, indicating that removals occurred prior to pollinaria deposition. The densities of the symbols (bars within a circle) represent larger sample size..

REPRODUCTIVE SUCCESS AND POLLINATOR LIMITATION.

As many studies have shown that orchids are pollinator limited (for an extensive review see Tremblay *et al.* 2005) we predicted that pollination would be lower in isolated plants. We found that fewer pollinaria were removed from flowers of isolated plants than from plants with close neighbors (0.11 vs 0.18), and that the effect of flower isolation on pollinaria deposition was less marked but still significant (0.02 vs 0.14). These results indicate differences in the effect of local inflorescence density on male and female functional success.

The ratio of pollinaria removed versus pollinaria deposited was approximately 1.5:1 in *E. brunonis*. This ratio is high relative to that reported for several other orchids (Tremblay *et al*. 2005). However, a similar ratio was observed in the Puerto Rican species *Ionopsis utricularioides* = 1.96:1 (Montalvo & Ackerman 1987), *Comparettia falcata* = 1.35:1

(Salguero-Faría & Ackerman 1999), *Satyrium bicorne* = 0.30:1 (Ellis & Johnson 1999) and *Aerangis verdickii* = 2:1-3:1 (Light *et al*. 2004). All of these species offer nectar rewards which are much higher deposition to removal ratio as compared to Central American species *Stelis argentata* and the Brazilian species *Bulbophyllum ipanemense*, where the ratios are, respectively, 26.7:1 (Christensen 1992) and 24.2:1 (Borba & Semir 1998). Once again, the observed visitation rate of 10–15% suggests that *E. brunonis* is not a deceptive flower or at least offers a higher attracting ability then most deceptive species.

Our results suggest that there is a high efficiency, averaged across all sites, of transfer of pollen to the stigmatic surfaces. Assuming that removal and deposition of pollinaria were recorded with equal accuracy, our data suggest that approximately 64% of all pollinaria removed were eventually deposited on conspecific stigmas (i.e. there would have been a ratio of three pollinaria removed for every two deposited on a stigmatic surface). At present we are ignorant of the identity of the pollinator, (other than it is a bee in the genus *Halictus)*, and of the mechanism by which the insects are apparently attracted. The ratio of pollinaria deposited to pollinaria removed suggests that the pollination mechanism is effective on those occasions when visitation occurs. Knowledge of the species pollinating *E. brunonis* might help to explain the high efficiency of pollination.

Since none of the populations behaved significantly differently from each other there is no evidence to suggest that the effect of density on reproductive success is population/site dependent. Moreover, wherever this orchid is present it appears to be a common component of the flora regardless of the extent of its bushland habitat (i.e. small fragmented areas of remnant habitat or of larger areas of intact habitat: A.P. Brown, 2006, unpublished data). The observed variation in population density at the sampled sites may not necessarily represent the range of densities in this species, since fire can affect flowering densities and the fire histories of particular sampled sites are unknown.

If the pattern of reproductive success observed in *Elythranthera brunonis* during this short survey is consistent over longer periods then *E. brunonis* is partially affected by the spatial organization of individuals. Clumps of individuals have higher reproductive success through both male and female measures. Local pollination dynamics may be affected by other factors that mask the pollinator behavior in relation to the flowering densities. Furthermore, the high efficiency of pollinations suggests that *E. brunonis* is not a deceptive species and the autecology's of this species should to be investigated in greater depth.

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LITERATURE CITED

Ackerman, J.D. 1989. Limitations to sexual reproduction in *Encyclia krugii* (Orchidaceae). Syst. Bot. 14: 101-109.

Ackerman ,J.D. 1990. Inflorescence size and fruit distribution among individuals of three orchid species. Amer. J. Bot. 77: 1378-1381.

Alexandersson ,R. & J. Ågren. 1996. Population size, pollinator visitation and fruit production in the deceptive orchid *Calypso bulbosa*. Oecologia 107: 533-540.

Borba E.L. & S. Semir. 1998. Wind-assisted fly pollination in three *Bulbophyllum* (Orchidaceae) species occurring in Brazilian campos rupestres. Lindleyana 13: 203-218.

Bower, C.C. 2001a. *Caladenia*: pollination. *In*: A.M. Pridgeon *et al.* (eds.). Genera Orchidacearum, 2; Orchidoideae 1. Oxford University Press. pp. 93-97.

Bower, C.C. 2001b. *Elythranthera*: pollination. *In*: A.M. Pridgeon *et al.* (eds.). Genera Orchidacearum, 2; Orchidoideae 1. Oxford University Press. pp. 103-104.

Brown, A.P. 1999. *Orchids of the South-West*. Department of Conservation and Land Management, Perth, Western Australia.

Calvo, R.N. 1990. Inflorescence size and fruit distribution among individuals of three orchid species. Amer. J. Bot. **77**: 1378-1381.

Christensen, D.E. 1992. Notes on the reproductive biology of *Stelis argentata* Lindl. (Orchidaceae: Pleurothallidinae) in eastern Ecuador. Lindleyana **7**: 28-33.

Dafni, A. & P. Bernhardt. 1990. Pollination of terrestrial orchids of southern Australia and the Mediterannean region: systematic, ecological and evolutionary implications. Evol. Biol. 24: 193-253.

Dixon, K.W., S.P. Kell, R.L. Barrett & P.J. Cribb (eds.). 2003. *Orchid Conservation.* Natural History Publications (Borneo), Kota Kinabalu, Sabah, Malaysia.

Dressler, R.L. 1993. Phylogeny and Classification of the Orchid Family. Cambridge University Press, Cambridge.

Ellis, A.G. & S.D. Johnson. 1999. Do pollinators deter-

mine hybridization patterns in sympatric *Satyrium* (Orchidaceae) species? Pl. Syste. Evol. 219: 137-150.

Firmage, D.H. & F.R. Cole. 1988. Reproductive success and inflorescence size of *Calopogon tuberosus* (Orchidaceae). Amer. J. Bot. 75: 1371-1377.

Fritz, A.L. & L.A. Nilsson. 1994.How pollinator-mediated mating varies with population size in plants. Oecologia 100: 451-462.

George, A.S. 1963. Notes on western Australian Orchidaceae. III. *Elythranthera*, a new genus for the enamel orchids. Western Australia Naturalist 9: 2-9.

Gill, D.E. 1996. The natural population ecology of temperate terrestrials: Pink lady's slippers, *Cypripedium acaule*. *In*: C. Allen (ed.). North American Native Terrestrial Orchids: Propagation and Production. North American Native Terrestrial Orchid Conference, Germantown, MD. pp 91-106.

Hoffman, N. & A. Brown. 1984. Orchids of South-West Australia. First Edition. University of Western Australia Press, Nedlands.

Hoffman, N. & A. Brown. 1992. Orchids of South-West Australia. Second Edition. University of Western Australia Press: Nedlands.

Hoffman, N. & A. Brown. 1998. Orchids of South-West Australia. Revised Second Edition with supplement. University of Western Australia Press: Nedlands.

Hopper, S.D. & A.P. Brown. 2001a. Contributions to Western Australian orchidology: 2. New taxa and circumscriptions in *Caladenia* (Spider, Fairy and Dragon Orchids of Western Australia). Nuytsia 14: 27-314.

Hopper, S.D. & A.P. Brown. 2001b. Contributions to Western Australian orchidology: 1. History of early collections, taxonomic concepts and key to genera. Nuytsia 14: 1-26.

Jacquemyn, H., R. Brys & M. Hermy. 2002. Flower and fruit production in small populations of *Orchis purpurea* and implications for management. *In*: P. Kindlmann, J.H. Willems & D.H. Whigham (eds). Trends and Fluctuations and Underlying Mechanisms in Terrestrial Orchid Populations. Backhuys Publishers, Leiden, the Netherlands. pp. 67-84.

Jersákova J., P. Kindlmann & M. Stritesky. 2002. Population dynamics of *Orchis morio* in the Czech Republic under human influence. *In*: P. Kindlmann, J.H. Willems & D.H. Whigham (eds). Trends and Fluctuations and Underlying Mechanisms in Terrestrial Orchid Populations. Backhuys Publishers, Leiden, the Netherlands. pp. 187-208.

Jersákova, J. & P. Kindlmann. 2002. Key factors affecting shoot growth and flowering performance of *Dactylorhiza fuchsii*. *In*: P. Kindlmann, J.H. Willems & D.H. Whigham (eds). Trends and Fluctuations and Underlying Mechanisms in Terrestrial Orchid Populations. Backhuys Publishers, Leiden, the Netherlands. pp. 99-113.

Jones, D.L. 1988. Native Orchids of Australia. Reed Books, Frenchs Forest, Australia.

Jones, D.L. 2001. Subtribe Caladeniinae. *In*: A.M. Pridgeon *et al.* (eds.). Genera Orchidacearum, 2; Orchidoideae 1. Oxford University Press. pp. 83-115.

Kores P.J., M. Molvray, P.H. Weston, S.D. Hopper, A.P. Brown A. P., K.M. Cameron & M.W. Chase. 2001. A phylogenetic analysis of Diurideae (Orchidaceae) based on plastid DNA sequence data. Amer. J. Bot. 88: 1903–1914.

Light M., H. Koopowitz & T. Marchant. 2003. The impact of climatic, edaphic and physiographic factors on the population behaviour of selected temperate and tropical orchids. *In*: K.W. Dixon, S.P. Kell, R.L. Barrett & P.J. Cribb (eds.). 2003. *Orchid Conservation*. Natural History Publications (Borneo), Kota Kinabalu, Sabah, Malaysia. pp. 357-382.

Meléndez-Ackerman, E.J. & J.D. Ackerman. 2001. Density-dependent variation in reproductive success in a terrestrial orchid. Pl. Syst. Evol. 227: 27-36.

Montalvo, A.M. & J.D. Ackerman. 1987. Limitations to fruit production in *Ionopsis utricularioides*. Biotropica 19: 24-31.

Pacala, S.W. & Jr. Silander. 1985. Neighborhood models of plant population dynamics I. Single–species models of annuals. Amer. Naturalist 125: 385-411.

Peakall, R. 1989. The unique pollination of *Leporella fimbriata* (Orchidaceae): pollination by pseudocopulating male ants (*Myrmeca urens*, Formicidae).Pl. Syst. Evol. 167: 137-148.

Piper, J.G. & S. Waite. 1988. The gender role of flowers of broad-leaved helleborine, *Epipactis helleborine* (L.) Crantz (Orchidaceae). Functional Ecology 2: 35-40.

Pridgeon, A.M., P.J. Cribb, M.W. Chase & F.N Rasmussen (eds). 2001. Genera Orchidacearum. 2. Orchidoideae, Part 1. Oxford University Press, Oxford.

Primack, R. & E. Stacy. 1988.Cost of reproduction in the pink lady's slipper (*Cypripedium acaule*, Orchidaceae): An eleven-year experimental study of three populations. Amer. J. Bot. 85: 1672-1679.

Sabat, A.M. & J.D. Ackerman. 1996. Fruit set in a deceptive orchid: the effect of flowering phenology, display size, and local floral abundance. Amer. J. Bot. 83: 1181-1186.

Salguero-Faría, J.A. & J.D. Ackerman. 1999. A nectar reward: is more better? Biotropica 31: 303-311.

Schemske, D.W. 1980. Evolution of floral display in the orchid *Brassavola nodosa*. Evolution 34: 489-493.

Snow, A.A. & D.F. Whigham. 1989. Cost of flower and fruit production in *Tipularia discolor*. Ecology 70: 1286-1293.

Stoutamire, W.P. 1974. Australian terrestrial orchids, thynnid wasps, and pseudocopulation. Amer. orch. Soc. Bull.

43: 13–18.
Stoutamire, W.P. 1975. Pseudocopulation in Australian terrestrial orchids.Amer. orch. Soc. Bull. 44: 226–233.
Stoutamire, W.P. 1981.Pollination studies in Australian terrestrial orchids. National Geographic Society Research Reports 13: 591–98.
Stoutamire, W.P. 1983. Wasp-pollinated species of *Caladenia* (Orchidaceae) in South-western Australia. Austral. J. Bot. 31: 383–394.
Tali, K. 1996. Spring-flowering and summer-flowering populations of *Orchis ustulata* L. (Orchidaceae) in Estonia; their comparison and distribution. J. Europ. Orch. 28: 573-582.
Tali, K. 2004. Species structure in *Neotinea ustulata*. PhD Thesis, University of Tartu, Tartu, Estonia.
Tremblay, R. L., J.D. Ackerman, J.K. Zimmerman & R. Calvo. 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. Biol. J. Linnean Soc. 84: 1-54.
Tremblay, R.L. 2005. Larger is better: The effect of floral display on reproductive success in two populations of *Caladenia* (*Stegostyla) gracilis* R. Br. Muelleria 22: 77-85.
Willem, J. 2002. A founder population of *Orchis simia* in the Netherlands. *In*: P. Kindlmann, J.H. Willems & D.H. Whigham (eds). Trends and Fluctuations and Underlying Mechanisms in Terrestrial Orchid Populations. Backhuys Publishers, Leiden, the Netherlands, pp. 23-33.
Zimmerman, J. K. & T.M. Aide. 1989. Patterns of fruit production in a neotropical orchid: pollinator vs. resource limitation. Amer. J. Bot. 76: 67-73.

Richard Bateman is an evolutionary biologist and palaeobiologist who has studied orchids for most of his life. His particular interests are morphological and molecular phylogeny reconstruction, species delimitation, and saltational explanations of major steps in evolution. He is currently a Research Associate at the Royal Botanic Gardens Kew, Head of Policy at the Biosciences Federation, Visiting Professor at the University of Reading, President of the Systematics Association and of the UK Hardy Orchid Society, and Vice President of the Linnean Society.

Andrew Brown is a conservation biologist and taxonomist who has studied orchids for much of his life. Andrew has authored numerous papers and publications on Western Australian orchids and in particular has been involved in naming over 150 new Western Australian taxa, has co-authored Orchids of South-west Australia and is currently preparing a new book covering all the Western Australian orchid species. Apart from the Orchidaceae Andrew is interested in the Mypoporaceae and has prepared text for a soon to be published field guide on *Eremophila*. He is currently the State Threatened Flora Coordinator based in the Western Australian Department of Environment and Conservation's Species and Communities Branch and, as such, coordinates research, recovery and other work on threatened Western Australian plants. Andrew is a life member of the Western Australian Native Orchid Study and Conservation Group, and a member of the Australian Orchid Foundation and Australasian Orchid Specialist

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PRACTICAL ORCHID CONSERVATION: INTEGRATED APPROACHES

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RESCUING *CATTLEYA GRANULOSA* LINDLEY IN THE WILD

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KEY WORDS: *Cattleya granulosa*, Brazil, habitat destruction, re-introduction, plant morphology

Introduction

The Orchid Society of Rio Grande do Norte State, Brazil, through its Group of Experimental Interactive Research (SORN/GEPI), has been studying the occurrence of *Cattleya granulosa* Lindley, 1842, specifically in the coastal sand plain and dune vegetation ("restinga") and in remaining patches of Atlantic Rainforest in the state. *C. granulosa* is threatened largely by urban development and, at a smaller scale, by plant collectors (Ferreira, 1992). It is, in theory, a biological indicator of reasonable importance for the evaluation of the ecological equilibrium in the sand plain vegetation dominated by *Eugeniacea* spp (Myrtaceae). *C. granulosa* is sensitive to urban development, climatic variations and to the attack of insects and mammals and its morphology reflects any of these aggressions. Mapping and characterizing the different populations of *C. granulosa* will provide additional information necessary for the continuation of existing programs of SORN/GEPI: (a) Project "Adopt an Orchid" (*Cattleya granulosa*), with the goal of stopping orchid collection in the wild and making cultivated seedlings available, and (b) Program "The *Cattleya granulosa* universe" directed at primary school and high school students. The latter program includes guided field trips with instruction about the sand dune ecosystem, distribution of *C. granulosa*, and the importance of its conservation, considering the interdependence between orchids and the environmental physical, chemical and biological characteristics. The main goal of this project is to characterize the habitats where this species grows, in order to make models for their introduction in other areas and to be able to protect them in areas of ecological stress.

Methods

Areas for study will be delimitated and plotted on a map (DHN number 23400) using GPS data. Soil samples will be collected in different *C. granulosa* habitats and sent for macro and micro nutrient analysis. Plant species associated with *C. granulosa* will be identified and, whenever necessary, they will be collected and dried, to make herbarium specimens. Five specimens of *Cattleya granulosa,* at each study site, will be marked and measurements will be made (number of pseudobulbs, estimated age, biometric parameters). Some will be dried to make herbarium specimens. Capsules will be collected from different sites, for sowing and germination of *C. granulosa,* using asymbiotic orchid culture techniques. Two to three-year-old seedlings will be introduced to the areas from where the seeds have been collected or in protected areas in the neighborhood.

Previous Results

All populations of *Cattleya granulosa* studied occurred on sand dunes covered by low vegetation, at a distance of 0.5 to 3 km from the beach (Fig. 1). All plants were found entangled with scrubs and bromeliads and most of them were growing on branches close to the sand (Fig. 2-4). Populations were mapped for 120 km, from lat. 06°10'30''S, long. 35°50'30''W to lat. 05°08'50''S, long. 35°34'19''W.

FIGURE 1. Sand dune low vegetation habitat near Natal, RN, Brasil.

FIGURE 2. *Cattleya granulosa* growing among shrubs.

FIGURE 3. *Cattleya granulosa* epiphytic on *Eugenia* sp (Myrtaceae).

FIGURE 4. *Cattleya granulosa* flowers appearing above the height of the vegetation patch.

Analysis of measurements made up to now, on 75 plants and a total of 300 pseudobulbs (the four last ones of each plant), showed a continuum in length variation from 4 cm to 97 cm, with up to 73% between 21-50 cm. Length (5 to 30 cm) and width (1.5 to 11 cm) of the larger leaves, as well as diameter of flowers (3 to 15 cm) also varied a lot among the sampled plants. Number of flowers per spike was between 1 and 18, although most of the plants analyzed had from one to five flowers per spike. Very few fruits were found in the field. Up to now no clear morphological distinction between the different populations has been found (Câmara Neto *et al*., 2005)

During the raining season (March to June) adult plants from different populations were reintroduced

FIGURE 5. Urban expansion in the "restinga" area.

FIGURE 6. "Orchid Woods" urban development in area to be deforested.

in a private ecological reserve in order to test their reliance to the process. One year after being transplanted all of them looked healthy and flowered. Rescue actions in areas to be used for urban developments and introduction of those plants in private land (ecological reserves or not) is continuing, although with many difficulties.

A few years ago one hundred seedlings of *Cattleya granulosa* were distributed among an interested public that registered themselves, as part of the sub-project "Adopt an orchid". On the occasion, 600 people applied to be entitled to grow one seedling of what people from Rio Grande do Norte State know as "the orchid". In 2007 the first group of people who were given seedlings will be visited for the following up of the project. Thousands of seedlings of different varieties of *C. granulosa* are growing in the lab.

Discussion

The expansion of urban boundaries and the construction of infrastructure for tourism in recent years (Fig. 5-6) have been added to traditionally destructive uses of the environment, making the "restinga" one of the most threatened ecosystems in Brazil. The "restinga" is considered part of the Atlantic Rainforest dominium and only 5-7% of this biome is still left. In the last two decades especially in the NE states of Brazil, there has been a boom of settlement of this almost flat plain near the coast, and the native vegetation of the "restinga", the only habitat of *Cattleya granulosa*, is disappearing quickly. Sometimes it is only a matter of a few days, or even few hours, for large areas of native vegetation to be razed to give way to legal "loteamentos" (subdivisions). This has been happening faster than the local orchid societies are able to act and rescue the plants.

On the other hand, *Cattleya granulosa*, which has flowers with a large color and pattern variation, is still very susceptible to orchid collectors. Most orchid collections in Rio Grande do Norte State have many varieties of the species and, as it is a species rarely available in commercial nurseries, collectors are often looking for another exclusive plant from the remaining patches of "restinga".

The SORN/GEPI group has now chosen a second private property, located in the natural environment of *Cattleya granulosa*, to reintroduce plants rescued from the surrounded area. As public protected areas in the state are rare and not well looked after, our goal is to create a network of private areas committed to conservation and that will maintain the genetic diversity of the species.

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LITERATURE CITED

Câmara-Neto, C., I. Chaves-Câmara& W. Prado-Martins. 2005. Contribuição ao Conhecimento da Ocorrência da *Cattleya granulosa* Lindley, 1842, na Vegetação de Restinga do Litoral do Rio Grande do Norte. Orquidário, 19 (2): 48-55.

Ferreira, A. 1992. Subsídios ao Projeto Granulosae. Recife, SOPE. 42pp.

Clementino Câmara-Neto is a chemist. For ten years he has been interested in orchids and especially in the conservation of *Cattleya granulosa*. He was one of the founders of the Group of Experimental Interactive Research and has participated on many projects in conservation of the species and its habitat, its reproduction and environmental education. He has produced a video and published several articles on the subject. His other area of research in orchids is with seaweed fertilizers.

Iuná Chaves-Câmara is a geographer and worked as a school teacher. Together with her husband Clementino, she was one of the founders of the Group of Experimental Interactive Research and has participated on many projects on conservation of the species and its habitat, its reproduction and environmental education.

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EFECTO DEL ACIDO INDOLBUTIRICO (IBA) Y 6-BENCILAMINOPURINA (BAP) EN EL DESARROLLO *IN VITRO* DE YEMAS AXILARES DE *ENCYCLIA MICROTOS* (RCHB.F.) HOEHNE (ORCHIDACEAE)

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ABSTRACT. *Encyclia microtos* (Rchb.f.) Hoehne, is an endemic orchid that are distributed in the north of Peru (Tumbes and Piura) and it is being depredy illegally causing the extinction of the same one. With interest to maintain his conservation, is an alternative by means of the propagation *in vitro*. For that reason the objective of this research is to evaluate the indolbutiric acid effect (AIB) and 6-bencilaminopurine (BAP) in the development in *vitro* of axillar bud of *E. microtos*. The experience with plant of 120 days of age began, it was introduced in medium MS (Murashige and Skoog) suplement with sucrose, phytagel, AIB , BAP (single or combined) and adjusting to pH of 5.5. Nine treatments were made, in a rank of 0; 1 and 2 ppm (single or combined). The results showed that BAP (2 ppm) promoted the greater development of axillary buds with a rate of multiplication of the double of plant to the 90 days in which they were exposed to the growth regulators. To single concentrations of AIB (1 ppm and 2 ppm), promoted a better development of leaves, roots and length of the same ones.

KEY WORDS: *in vitro* axillar bud, *Encyclia microtos*, AIB, BAP

Introducción

La familia Orchidaceae, está constituido aproximadamente por 17000 a 35000 especies (40% de las monocotiledóneas) y es la más extensa de las angiospermas, ocupan un amplio rango de los hábitats ecológicos, son las más evolucionada debido a que han desarrollado un gran potencial morfogenético, fisiológico y estructural, debido a muchas estructuras reproductivas condicionadas por la alta totipotencialidad de sus células. Estos sistemas reproductivos difieren en gran medida en diferentes zonas de temperatura, clima, etc, las cuales permite adaptarse y determinan su estrategia de supervivencia, lo cual explica su amplia área de distribución (Rasmussen 1985, Dressler 1993, Batygina *et al*. 2003).

El género *Encyclia* fue establecido por Hookern en 1828, son generalmente epífitas, endémicas del continente americano, se extiende desde el sur de Méjico hasta la parte norte de América del Sur. *Encyclia microtos* (Reichb.f.) Hoehne 1952, es una especie epífita con un período floral de diciembre a febrero, es endémica del sur del Ecuador y el norte del Perú, desde el bosque seco de Tumbes hasta el bosque de Cuyas en Piura. (Bennett & Christenson 1993).

De acuerdo a los estudios de Bennett y Christenson (1993), Juergen *et al*. (1996), Díaz (2003) y Fernández (2002) el Perú tiene alrededor de tres mil especies de orquídeas, distribuidas de Tumbes a Puno. La mayor diversidad se concentra en la ceja de selva, comprendida entre los 500 y 3,600 m.s.n.m. La menor diversidad corresponde a la selva baja (entre los 300 m.s.n.m.) y la serranía entre los 2,600 y 3,600 m.s.n.m. Más de la mitad de las orquídeas son epífitas, las cuales contribuyen con el 11% de las 17 mil especies de angiospermas peruanas. Se calcula aún que existen muchas más especies nuevas sobretodo en las zonas remotas e inaccesibles del país así como en las cordilleras aisladas. Las orquídeas denotan un alto grado de endemismo lo cual los hace más vulnerables debido a la destrucción de sus hábitats por la quema y tala de los bosques de la agricultura itinerante.

Desde el desarrollo del método de germinación asimbiótica en semillas de orquídeas por Knudson (1946), las técnicas de cultivo han sido usadas para la

propagación a gran escala de muchas especies de orquídeas y sus híbridos (Rao 1977, Arditti & Ernst 1993). Esto ha llevado a muchas firmas comerciales a usar el sistema *in vitro* por la rapidez de su propagación masiva (Teixeira 2003). Estas técnicas han mostrado resultados poco exitosos en nuestro país, limitándose a la extracción y comercialización directa en el campo, lo cual reduce significativamente el número de éstas en su hábitat, por lo tanto es necesario establecer pautas muy claras y precisas acerca de su conservación o manejo sostenible, puesto que constituyen uno de nuestros patrimonios nacionales (Morel 1974, Teng *et al.* 1997, Torres & Mogollón 2000, Decrose *et al*. 2003, Calatayud 2004, Puchooa 2004),

Las auxinas participan en muchos procesos del desarrollo vegetal: crecimiento, dominancia apical, enraizamiento, partenocarpia, tropismos, abscisión, elongación celular y frecuentemente en embriogénesis y organogénesis en cultivos en suspención. Uno de los reguladores más utilizados en la formación de raíces es el ácido indolbutírico (IBA) debido a que no causa efectos fitotóxicos, ni inhibe el crecimiento caulinar (Pierik 1990, Salisbury & Ross 2000).

Las citoquininas estimulan la división y diferenciación celular que afecta a una amplia gama de procesos de crecimiento y desarrollo de las plantas, entre lo que cabe citar la iniciación del desarrollo del cloroplasto, fotosíntesis, la expansión de los cotiledones, así como la inducción de la formación de brotes en cultivo vegetales. (Salisbury & Ross 2000, Ascón-Bieto & Talon 2001, Martinez 2002).

El uso de reguladores de crecimiento como alternativa para el incremento de la proliferación de brotes directos han probado la influencia de diferentes dosis de auxinas y citoquininas en el medio de cultivo basal Murashige y Skoog (1962) en diferentes géneros como *Cattleya aurantiaca (*Mauro 1994, Torres & Mogollón 2000, Krapiec *et al*. 2003), *Cymbidium aloifolium*, *Dendrobium aphyllum* y *Dendrobium moschatum* (Nayak *et al*. 1997), *Phalaenopsis* sp (Kosir *et al*. 2004), *Vanda spathulata* (Decrose *et al*. 2003), *Catasetum fimbriatum* (Peres *et al*. 2001), entre otros.

Nuestras especies nativas de orquídeas, amenazadas o en peligro de desaparecer, pueden protegerse utilizando las técnicas de propagación masiva *in vitro*, siempre y cuando se asegure su reproducción en cantidades adecuadas. Las plantas propagadas podrían distribuirse a viveros comerciales, lo que produciría una disminución de la extracción ilegal en sus hábitats naturales; asimismo, podrían ser reintroducidas a los hábitats en los que ha desaparecido completamente (León, 1995). El interés por las orquídeas si bien son apreciadas por sus vistosos colores, formas de sus flores y por la durabilidad de las mismas; son plantas que requieren de condiciones especiales para la germinación de las semillas y de períodos vegetativos largos antes de su floración, las cuales son necesarias diferentes técnicas para su propagación. Conociendo esta realidad, el objetivo de este trabajo es reconocer el efecto de ácido indolbutírico (IBA) y BAP en el desarrollo de yemas axilares en plántulas de *Encyclia microtos* (Reichb.f.) Hoehne (Orchidaceae).

Material y métodos

MATERIAL BIOLOGICO. La especie *E. microtos* fue determinada en el Herbarium Truxillense (HUT) de la Universidad Nacional de Trujillo (Perú). La experiencia se desarrolló en el laboratorio de Fisiología y Cultivos de Tejidos Vegetales de la Universidad Nacional de Trujillo, las cuales proporcionó las plántulas de *E. microtos* (fig. 1) en condiciones *in vitro* y de 120 días de edad (fig. 2).

DISEÑO EXPERIMENTAL. El medio nutritivo basal usado fué el de Murashige y Skoog (MS) (1962), vitaminas como mio-inositol 20 gl^{-1}; ácido nicotínico 0,1 gl^{-1}; piridoxina-HCl 0,1 gl^{-1}; tiamina-HCl 0,1 gl^{-1}; glicina 0,1 gl^{-1}; las que fueron suplementadas con IBA y bencilaminopurina (BAP), sacarosa (3 %), fitagel (0,25 %) y ajustándose a un pH de 5,5. Los diferentes tratamientos tuvieron las siguientes combinacines de IBA y BAP:

	Reguladores de crecimiento
Tratamientos	**IBA x BAP**
T1	0.0 x 0.0
T2	1.0 x 0.0
T3	2.0 x 0.0
T4	0.0 x 1.0
T5	1.0 x 1.0
T6	2.0 x 1.0
T7	0.0 x 2.0
T8	1.0 x 2.0
T9	2.0 x 2.0

FIGURA 1. Plántulas *in vitro* de *E. microtos* de 120 días de edad.

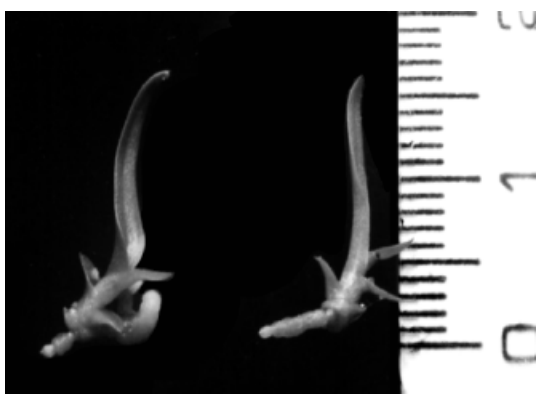

FIGURA 2. Plántulas de *E. microtos* usados como explantes que fueron expuestos a diferentes concentraciones de IBA y BAP, para inducir el desarrollo de yemas axilares.

El volumen de 2 ml de medio de cultivo se colocó en frascos de vidrio, las cuales fueron sellados con papel de aluminio, y posteriormente autoclavado a 120^{O}C por 15 minutos.

Se utilizó una cámara aséptica para realizar el proceso de introducción de las plántulas en los frascos de vidrio (una plántula por frasco, diez plántulas por tratamiento), luego se llevaron a la sala de incubación y fueron colocados en un estante metálico donde recibieron fuente luminosa proporcionada por tres fluorescentes de luz blanca (40 w) y con un fotoperíodo de 16:8 y una temperatura promedio de 25^{O} C.

La evaluación se realizó cada 30 días durante tres meses y se anotaron los siguientes datos: presencia y número de desarrollo de yemas axilares, número de hojas, número y longitud de raíces las cuales fueron sometidos a análisis estadísticos: promedios, análisis de varianza, tasa de multiplicación y prueba de significación Tukey con un nivel de significancia de 0,05.

Resultados

Todos los tratamientos ensayados, mostraron diferencias significativas (ANAVA, significancia 95%), y el mejor tratamiento resultó ser BAP (2 ppm) comprobado con la prueba de rangos estandarizados de Tukey con un nivel de significancia al 95% (tablas 1 y 2).

El desarrollo de yemas axilares fueron a los 30 días de ser expuestas a los reguladores de crecimiento. Se observó un mayor desarrollo de yemas axilares a concentraciones de 2 ppm de BAP obteniéndose en promedio el doble de plántulas, a la vez también se observó un mayor desarrollo de 1.8 plántulas concentraciones combinadas de 2 ppm de IBA con 2 ppm de BAP durante los 90 días expuestos a los reguladores de crecimiento y un menor desarrollo a 2 ppm de IBA aumentando sólo a 1.13 plántulas desarrolladas en promedio (fig. 3).

TABLA 1. Análisis de varianza para el número de brotes obtenidos a partir de yemas axilares de *E. microtos* a los 90 días de ser sometidas a diferentes concentraciones de IBA y BAP.

F de V	GL	Sum. de Cuad	Cuad. Med	Coef. F	P-valor
AIB	2	1.17037	0.5851850.	16	0.3178
BAP	2	6.05926	3.02963	5.99	0.0033*
AIB x BAP	4	2.6963	0.674074	1.33	0.2615
Residuos	126	63.7333	0.50582		
Total	73.6593	134			

* P-valor inferior a 0.05 (BAP), efecto estadísticamente significativo en el número de brotes para un 95. 0 %.

TABLA 2. Tukey para el número de yemas axilares de *E. microtos* según BAP.

BAP	diferencias	+/- límites
1 ppm	-0.33333	0.355617
2 ppm	* -0.51111	0.355617
BAP x IBA	-0.17777	0.355617

Diferencia significativa para el número de brotes a un nivel de significancia de 95.0%.

Un mayor aumento a 1.8 hojas por plántula en promedio, se observó a 2 ppm de IBA en comparación con los demás tratamientos en que no se observó una diferencia significativa (fig. 4).

En relación al número de raíces se observó un incremento de 2.56 raíces a 1 ppm de IBA y un 2.29 raíces a 2 ppm de IBA y menos efectivo fue a concentraciones combinadas de 1 ppm de IBA con 1 ppm de BAP aumentando sólo 1.18 raíces en promedio (fig. 5).

Para la longitud de raíces hubo un mayor desarrollo a concentraciones solas de 1 ppm y 2 ppm de IBA aumentado a 4.19 mm y 3.65 mm en promedio respectivamente. En los demás tratamientos se observó una menor desarrollo en la longitud de raíz se observó a 2 ppm de BAP aumentando sólo 0.39 mm (fig. 6).

Discusión

Los resultados obtenidos muestran que a 2 ppm de BAP, desarrollaron un mayor número de yemas axilares, en comparación a las otras concentraciones (fig. 3). Esto concuerda con Kosir *et al*. (2004), que reporta un mayor desarrollo de yemas a concentraciones altas de BAP en *Phalaenopsis* sp. usando nudos como explantes. Este tipo de regeneración podría mejorar la multiplicación masal a niveles comerciales, ya que reduce signi-

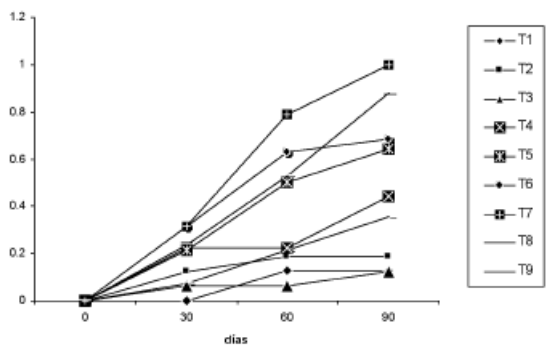

FIGURA 3. Número de yemas axilares en plántulas de *E. microtos* a los 120 días de edad y sometidos a diferentes concentraciones de IBA y BAP.

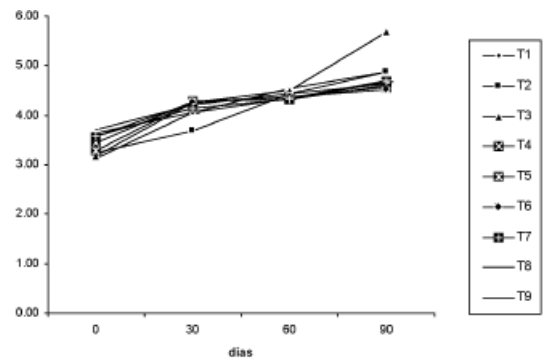

FIGURA 4. Número de hojas en plántulas de *E. microtos* a los 120 días de edad y sometidos a diferentes concentraciones de IBA y BAP.

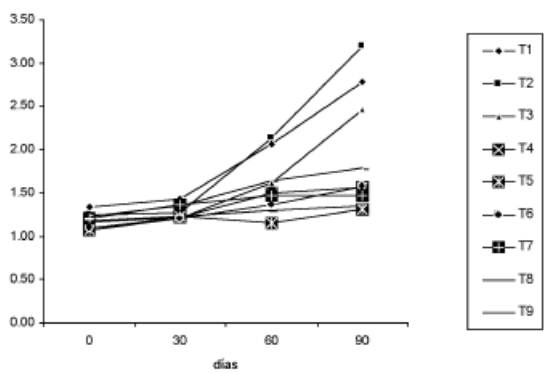

FIGURA 5. Número de raíces en plántulas de *E. microtos* a los 120 días de edad y sometidos a diferentes concentraciones de IBA y BAP.

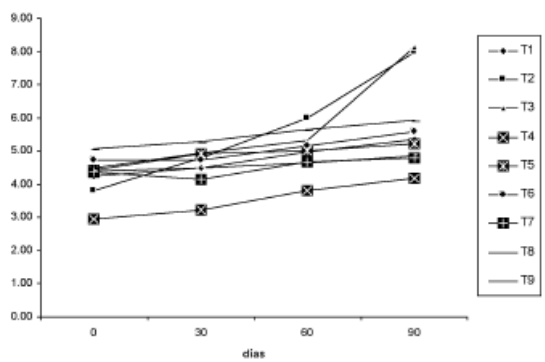

FIGURA 6. Longitud de raíz en plántulas de *E. microtos* a los 120 días de edad y sometidos a diferentes concentraciones de IBA y BAP.

ficativamente la variabilidad somaclonal originado por la formación de callos. Pierik (1990), sostiene que BAP a mayores concentraciones estimulan la división celular propiciando el crecimiento y desarrollo de nuevos tejidos, promoviendo la formación de yemas axilares, ya que disminuye la dominancia apical, iniciando así una descendencia homocigota idéntica a la planta madre. Además, esta citoquinina (BAP), regula muchos aspectos en el desarrollo de las plantas, incluyendo la división celular, neoformación de órganos, desarrollo de cloroplastos y senescencia (Ascon-Bieto & Talon 2001).

A concentraciones combinadas de 2 ppm de IBA y 2 ppm de BAP (T9), se observó un mayor número de brotes axilares, debido al efecto sinérgico entre estos dos reguladores (Pierik 1990, Hurtado & Merino 1994), Estos resultados coinciden con Krapiec *et al*. (2003), quienes lograron incrementar gradualmente un número mayor de brotes directos en *Cattleya walkeriana*, usando las mismas concentraciones de IBA y BAP en 90 días de estudio. Estos datos obtenidos estarían relacionados a un desencadenamiento fisiológico de la acción hormonal, condicionado por la aplicación e interacción de los reguladores de crecimiento exógenos, por las dosificaciones de las hormonas al medio, como también por la capacidad de absorción de los tejidos por difusión, como lo reporta Chico *et al.*, (2002), en segmentos nodales de *Vitis vinifera* var. "borgoña" expuestos a diferentes concentraciones de BAP y ácido naftalenacético (ANA).

La biosíntesis, conjugación, oxidación, transporte y efectos de auxinas y citoquininas sobre el órgano de crecimiento, se postula que son las raíces las principales zonas de biosíntesis de citoquininas y las exporta hacia los brotes, donde se promueve su desarrollo (caulogénesis). Inversamente, los brotes son considerados como la principal fuente de biosíntesis de auxinas, lo cual pueden ser inactivados por la conjugación y/o oxidación, tan bien como son exportadas hacia las raíces. Los brotes derivados de auxinas puede promover la iniciación y crecimiento de raíces (rizogénesis) (Peres *et al*. 2001), promoviendo a la vez la dominancia apical, la cual ejerce el control del ápice caulinar sobre el crecimiento de las yemas laterales. Se postula que el ápice caulinar reprime el crecimiento de las yemas axilares actuando como un sumidero, activado por la auxina, para la citoquininas derivadas de la raíz, lo que limitaría el transporte de las citoquininas hacia las yemas laterales (Ascon-Bieto & Talon 2001). Como las raíces de las orquídeas epífitas tienen algunos aspectos característicos, por ejemplo, la acumulación de clorofila y geotropismo negativo, las cuales pueden tener una considerable capacidad de biosíntesis de auxina, esto se observó que a concentraciones de 1 ppm y 2 ppm de IBA presenta un menor número de desarrollo de yemas axilares y mayor formación de raíces. Este desbalance, entre la formación de vástagos y raíces con concentraciones de citoquininas o auxinas, lo explica Peres *et al*. (2001), en plantas transgénicas expresando el gen *ipt* de *Agrobacterium tumefaciens*, lo cual presenta un aumento en la biosíntesis de citoquininas, en consecuencia, incrementó la formación de vástagos y la inhibición en la iniciación de raíces; contrariamente en plantas transgénicas expresando los genes biosintéticos de auxinas (*iaaM* y *iaaH*), tendieron a presentar una sustancial formación de raíces y la inhibición en el desarrollo de brotes axilares.

Salisbury y Ross (2001) resalta que el IBA se utiliza para causar la formación de raíces aún más a menudo que ANA o cualquier otra auxina. El IBA es activo pese a que se metaboliza con rapidez a IBA-aspartato y al menos otro compuesto conjugado con un péptido. Se ha sugerido que la formación de conjugado almacena al IBA y que su liberación gradual mantiene niveles adecuados de concentración de IBA, especialmente es las etapas finales de la formación de la raíz. Esto se comprueba con la figura 4, 5 y 6, en las cuales, el uso de IBA, promueve el enraizamiento y desarrollo de hojas a concentraciones de 1 ppm y 2 ppm, sin combinar ya que induce la elongación celular en las células de las raíces y promueve la dominancia apical. Sin embargo, BAP solo o combinado ofrece un efecto inhibitorio en la elongación de la raíz como lo reporta Collie y Kerbauy (1993) en *Catasetum* sp.

y otras especies de orquídeas. Este desarrollo vegetativo efectivo en las plántulas usando IBA como regulador de crecimiento coincide con Krapiec *et al.* (2003) en plántulas de *Cattleya walkeriana*, obteniéndose plántulas morfológicamente similares a las plantas adultas.

La mayoría de especies de orquídeas tienen la capacidad de propagación vegetativa, variando en los patrones de reproducción, esto se evidencia desde los estadios de protocormo, germinación de yemas apicales y axilares incluyendo las dormantes, órganos axilares como los rizomas, además los tallos, hojas, inflorescencias que también son usadas como explantes (Batygina *et al.* 2003, Puchooa 2004).

Algunos autores han usado nudos con yemas como explantes combinando incluso BAP y auxinas como ANA para aumentar la proliferación de brotes en *Cattleya aurantiaca* (Mauro *et al*. 1994), thidiazurón (TDZ) y ANA en *Cymbidium* aloifolium, *Dendrobium aphyllum* y *Dendrobium moschatum* (Nayak *et al*. 1997), bencilaminoadenina (BA) y ácido indolacético (AIA) en *Vanda spathulata* (Decrose *et al*, 2003), AIA y zeatina (Z) en *Catasetum* sp. (Peres *et al*., 2001), y usando solamente TDZ en ápices caulinares en *Cattleya mossiae* y la proliferación de brotes directos sin la formación previa de callos. La regeneración directa sin la indeseable formación de callos acorta el período de tiempo necesario para la regeneración y reducción de la posible incidencia de variación somaclonal (Kosir *et al.* 2004). Sin embargo, los mayores logros *in vitro* de orquídeas ha sido más frecuente en la proliferación de cuerpos protocórmicos a partir de callos usando diferentes partes de la planta como explantes en diferentes combinaciones de reguladores de crecimiento (Arditti & Ernst 1993. De Pauw *et al*. 1995. Teng *et al*. 1997, Young *et al*. 2000. Tokuhara & Mii 2001. Chen *et al.* 2002. Chen & Chang 2002. Puchooa 2004, Nagaraju & Mani 2005).

El establecimiento de esta simple técnica efectiva de propagación directa de yemas, evitando la variación somaclonal, podría mejorar la producción a escala comercial o también para la conservación *ex situ*, creando bancos de germoplasma de especies amenazadas en un corto período de tiempo en especies de orquídeas.

FIGURA 7. Planta madre (a) de *E. microtos* con el desarrollo de 2 yemas axilares (b y c) a los 90 días expuestos a BAP.

Conclusiones

BAP a concentraciones de 2 ppm y combinados IBA (2 ppm) con BAP (2 ppm), fueron los que promovieron el mayor desarrollo de yemas axilares obteniéndose una tasa de multiplicación del doble de plántulas y 1.8 plántulas respectivamente .

IBA a concentraciones de 1 ppm y 2 ppm, promovieron un mejor desarrollo de hojas (1.8 hojas en promedio a 1 ppm), formación de nuevas raíces (2.56 y 2.29 raíces en promedio a 1ppm y 2 ppm respectivamente) y longitud de raíces (4.19 mm y 3.65 mm en promedio a 1 ppm y 2 ppm respectivamente) en *E. microtos* (fig. 7).

LITERATURA CITADA

Arditti, J. & R. Ernst. 1993. Micropropagation of Orchids. John Wiley and Sons. New York.

Ascon-Bieto, J. & M. Talon. 2001. Fundamentos de Fisiología Vegetal. Edit. Interamericana. Mc Graw-Hill. Madrid

Batygina, T., E. Bragina & V. Vasilyeva. 2003. The Reproductive System and Germination in Orchids. Acta Biologica Cracoviensia. Series Botanica 45 (2): 21-34.

Benett, D. & E.A. Christenson. 1993. Icones Orchidacearum Peruvianum. Plates 1-200.

Kafkafi, U (ed.). Plant roots the hidden half. New York, Marcel Dekker. p. 875-894.

Calatayud, G. 2004. Diversidad de Orchidaceae en los bosques de la provincia San Ignacio (Cajamarca). Libro de Resúmenes del X Congreso Nacional de Botánica. Trujillo, Perú.

Chen, L., J. Chen & W. Chang. 2000. Efficient production of protocorm-like bodies and plant regeneration from flower stalk explants of the sympodial orchid *Epidendrum radicans*. In Vitro Cell. Dev. Biol. Plant 38: 441–445.

Chen, J. & W. Chang. 2002. Effects of tissue culture conditions and explant characteristics on direct somatic embryogenesis in *Oncidium* 'GowerRamsey'. Plant Cell Tissue. Organ Cult. 69: 41–44.

Chico, J., L. Cerna, R. Veneros, B. Valentin & M. Bobadilla. 2002. Propagación *in vitro* de *Vitis vinfera* var. borgoña. Rebiol 22 (1-2): 1-6.

Collie, S. & G. Kerbauy. 1993. Direct root tip conversion of *Catasetum* into protocorm-like bodies. Effect auxin and cytokinin. Plant Cell, Tissue and Organ Culture. 33: 39-44.

De Pauw, M., W. Remphrey & C. Palmer. 1995. The Cytokinin Preference for in vitro germination and protocorm growth of *Cypripedium candidum*. Annals of Botany. 75: 267-275.

Decrose, W., A. Gangaprasad, S. Seeni & S. Menon. 2003. Micropropagation and ecorestation of *Vanda spathulata*, an exquisite orchid. Plant Cell, Tissue and Organ Culture. 72: 199-202.

Díaz, M. 2003. Orquídeas del bosque de Cuyas (Ayabaca), Piura-Perú. Tesis para optar el Título de Biólogo. Universidad Nacional de Piura. Perú

Dressler, R.L. 1993. Phylogeny and classification of the orchid family. Portland, Oregon, Dioscorides Press: 314-318.

Fernández, R. 2002. Estudio de Orquídeas del Perú. Libro de Resúmenes de IX Congreso Nacional de Botánica. Pag 86. Universidad Nacional de la Amazonía.

Hurtado, D. & M. Merino. 1994. Cultivo de Tejidos Vegetales. Edit. Trillas. Mexico.

Juergen, N., L. Ibisch & W. Barthlott. 1996. Biodiversidad de epífitas – una cuestión de escala. Revista Jardín Botánico Nacional.

Kosir, P., S. Skof & Z. Luthar. 2004. Direct shoot regeneration from nodes of P*halaenopsis* orchids. Acta Agriculturae Slovenica, 83(2): 233 – 242.

Krapiec, P., M. Milaneza & F. Pires. 2003. Effects of different combinations of growth reguladors for bud induction from seedlings of *Cattleya walkeriana* Gardner (Orchidaceae). Acta Scientiarum: Biological Sciences. Maringá, 25(1): 179-182. Brasil

León, M. 1995. Conservación de especies peruanas de orquídeas utilizando la técnica de cultivos *in vitro*. Tesis para optar el título de Biólogo. Universidad Agraria La Molina. Lima. Perú

Martinez, E. 2002. Citoquininas y fitocromos. VIII Simposio. Metabolismo y modo de acción de Fitohormonas. Edit. Junta de Andalucía: Consejería de Agricultura y Pesca. Sevilla. España. pag. 173-182

Mauro, M. 1994. Influence of bencilaminopurine and alpha-naphthaleneacetic acid on multiplication and biomass production of *Cattleya aurantiaca* shoot explants. Lindleyana, West Palm Beach, 9: 169-173.

Morel, G., 1974. Clonal multiplication of orchids. *In*: C.L. Withner (ed.), The Orchids: Scientific Studies, pp. 169–222. Wiley, New York.

Murashige, T. & F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissues cultures. Physiol. Plant. 15: 473-497.

Nagaraju V. & S. K. Mani. 2005. Rapid In Vitro Propagation of Orchid *Zygopetalum intermedium*. Plant Biochemistry & Biotechnology. 14: 27-32.

Nayak N., S. Rath & S. Patnaik. 1997. In vitro propagation of three epiphytic orchids, *Cymbidium aloifolium* (L) Sw., Dendrobium aphyllum (Roxb.) Fish. and *Dendrobium moschatum* (Buch-Ham) Sw. through thidiazuron – induced high frecuency shoot proliferation. Scientia Horticulturae 71: 243-250.

Peres, L., N. Majerowicz & G. Kerbauy.2001. Dry matter partitioning differences between shoots and roots in two contrasting genotypes of orchids and their relationship with endogenous levels of auxins, cytokinins and abscisic acid. Revista Brasileira de Fisiologia Vegetal, 9: 169-176.

Peterson, R.L, Y. Uetake & C. Zelmer. 1998. Fungal symbioses with orchid protocorms. Symbiosis 25: 29-55.

Pierik, R. 1990. Cultivo in vitro de plantas superiors. Editorial Mundi Prensa. Madrid.

Puchooa, D. 2004. Comparison of Different Culture Media for the *In Vitro* Culture of *Dendrobium* (Orchidaceae). International Journal of Agriculture & Biology, 6 (5): 884-888.

Rao, A.N. 1997. Tissue culture in orchids industry. *In*: Bajaj, Y. P. S., Reinert J. Applied and Fundamental Aspects of Plants, Cell, Tissue and Organ Cultura. Springer-Verlag. Berlin. pp. 44-69.

Rasmussen, F. 1985. In The families of monocotyledons: Structure, evolution and taxonomy (RM Dahlgrn, H Clifford, PF Yeo, Editors), pp 249.

Salisbury, F. & C. Ross. 2000. Fisiología de las plantas. Thomson Edited Spain Paraninfo. Madrid.

Teixeira, J. 2003. Thin Cell Technology in ornamental plant micropropagation and biotechnology. African Journal of Biotechnology Vol. 2 (12), pp. 683-691.

Teng W., L. Nicholson & M. Teng. 1997. Micropropagation of *Spathoglottis alicata*. Plant Cel

Report. 16: 831-835.

Tokuhara K. & M. Mii. 2001. Induction of embryogenic callus and cell suspension culture from shoot tips excised from flower stalk buds of *Phalaenopsis* (orchidaceae). In Vitro Cell. Dev. Biol. Plant 37: 457-461.

Torres J. & N. Mogollón. 2000. Micropropagación de *Cattleya mossiae* Parker ex Hook mediante brotación axilar inducida por tidiazurón. Bioagro 12(1): 10-14.

Young P., H.N. Murthy & P. Yoeup. 2000. Mass multiplication of protocorm-like bodies using bioreactor system and subsequent plant regeneration in *Phalaenopsis.* Plant Cell, Tissue and Organ Culture 63: 67–72.

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THE CONSERVATION OF MADAGASCAR'S ORCHIDS . A MODEL FOR AN INTEGRATED CONSERVATION PROJECT

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Introduction

The fauna and flora of the large island of Madagascar, situated in the eastern Indian Ocean off the coast of East Africa, has been recognised for many years as one of the most peculiar anywhere in the world, and as one of the most endangered. It has a rich orchid flora of about 1000 species. Over 90% of its orchid species are endemic to the island and nearly 20% of its genera. Endemicity of its orchids at both the species and generic level is higher that anywhere else in the world. Many orchids are threatened by the rapid rate of environmental change in the island.

The joint Royal Botanic Gardens Kew / Parc Tzimbazaza Threatened Plants Programme, supported by the Ministry of Natural Resources and Forestry and by the University in Antananarivo, has adopted an innovative and integrated approach to plant conservation, using orchids, palms and succulents as its target groups. The starting point for the programme has been the production a new and detailed vegetation map of the island which pin-points all the vegetation types, including those that are not currently protected. This has been adopted by the government to earmark new reserves and protected areas. Orchid distributions, past and present, can be located on the map in order to see if species currently enjoy a degree of protection within reserved areas.

Six flagship orchid species have been used to raise local awareness of the threats to native orchids through an out-reach and poster programme in schools. Extensive field studies have established the distribution, numbers and genetic diversity of the target species. Seedlings have been raised *in situ* for eventual re-introduction and to provide nurseries with legitimate sources of rare species. The ecology of areas selected for re-introduction has been assessed. Planned re-introduction will only be undertaken when the appropriate monitoring programmes have been established.

Orchids are found in almost every environment in the island, and many species are threatened with extinction because of the rapid and extensive destruction of natural habitats by agriculture, mining and fire. A Field Guide to the orchids of the island is in preparation which should enable the orchids to be identified and named in the wild, an aid to the utilisation of orchids as a means of rapid assessment of the plant diversity and environmental health of habitats. This will be particularly useful since most modern surveys, relying on zoological rather than overall biological diversity, may well provide an inaccurate assessment of the value of a habitat for conservation purposes.

Madagascar

The Indian Ocean island of Madagascar lies about 400km from the east coast of Africa, the tropic of Capricorn running through the south and the northern tip pointing towards the equator. At 587,000 km^2 it is the fourth largest island in the world and larger than California and Oregon combined. It is thought that the first inhabitants arrived from Indonesia and Malaya between 1500 and 2000 years ago followed by migration from mainland Africa and elsewhere. Today it has a population of about 16 million people and almost as many zebu cattle, it is not a rich country. Traditional exports include minerals, prawns, vanilla and lychees but major investments are being made in new industries. Tourism, especially eco-tourism, is growing but still a relatively small money earner for the country.

The island has a great variety of bedrock and soil types as well as wide topographical variation, ranging

from tropical coastal forest to several mountain ranges over 2000m. It separated from the mainland about 165 million years ago as a result of continental drift and, in this way, a largely endemic flora and fauna developed, there being an exceptional level of endemism. An estimated 9500 different vascular plants, 81% of them endemic, are found there; at the last count 980 different orchids in 59 genera have been recorded, suggesting that over one in ten vascular plants on Madagascar are orchids. The endemic flora and fauna has fascinated explorers for centuries but our knowledge of it is still surprisingly limited.

Vegetation zones

The island has been divided into a number of fairly well defined areas of vegetation and climate, but more recently detailed mapping techniques, such as satellite imaging, have been used to re-define some of these zones. Innovative work by Du Puy & Moat (2003) and Moat (2005; www.vegmad.org), for example, matched underlying geology with vegetation. In addition, detailed analysis of the natural history of Madagascar has been published by a large group of authors in Goodman & Benstead (2003), giving further insight in the island's natural diversity. The following is a summary of the distinct areas of vegetation on Madagascar, it will also illustrate the delicate ecology of some representative orchids.

The eastern rain forest

This vegetation zone, covers a large part of the island and occurs from the north down the east to the south-east corner of the island. It runs from the coast to the crest of mountains lying parallel to the eastern length of the Island. This part of the country is under the influence of the South-east trade winds, which promote cloud formation and heavy rainfall throughout the year. Average annual rainfall is over 2000mm, topping 6000mm in parts of the Masoala peninsula in the north-east. There is no marked dry season at lower elevations and only a brief one higher up where it is cooler so that broad-leaf evergreen forest is supported. Temperatures are generally high with mean winter readings in the coolest season ranging from 18°C at sea level to 10°C at the top of the escarpment. Primary vegetation is mostly lowland rain forest with tall forest trees (up to 25-30m) but there are several distinct strata in the forest and a diffuse understory. This type of forest is rich in species. It is in much better condition in the north and north-east than further south where just a patchwork remains. This type of vegetation continues in the north-west around the Sambirano river valley where there is a short dry season and about 1800mm of rainfall. In cultivation plants of this area will need to be kept fairly moist throughout most of the year, the amount of heat and light required will vary much from species to species. *Cymbidiella flabellata*, for example, grows as a terrestrial in boggy areas and is sometimes seen in great quantity, plants can be fully exposed to sunlight near the sea but it can also be found at the edge of forest or beneath ericaceous scrub (*Philippia*). On the other hand *Cymbidiella falcigera* almost invariably grows on *Raphia* palms in marshy ground, the root system of the orchid is very elaborate and runs deeply into the soft bract fibre of the palm. This species is very difficult to establish in cultivation. The third species of this endemic genus is *C. pardalina* which lives in association with *Platycerium madagascariense*, a staghorn fern with net-veined sterile fronds. This association seems essential, at least in nature, for the development of the orchid. The roots of the orchid develop in the crown of sterile fronds of the fern that form a protective shell against direct sunlight and desiccation. Ants also often establish themselves in the crown of fronds of the fern. The orchid and fern live epiphytically up to 20m high on tree trunks, most commonly *Albizia*, this whole combination forming a very complex and vulnerable micro-ecosystem.

The eastern forest is the home of numerous orchids, the following are just a selection of the more unusual ones. *Beclardia macrostachya* often grows in semi-shade, while from the same habitat comes *Oeonia rosea* with its colourful lip. *Angraecum rubellum* is a twig epiphyte, the flowers are only a few millimetres but the pink colouration makes it very unusual. *Microcoelia gilpinae* is another colourful angraecoid; it is leafless and grows in the deep shade entwined amongst twigs. *Bulbophyllum* is well-represented as a genus with about 200 different species; one gem growing in deep shade and embedded in moss is *Bulbophyllum analamazoatra*. *Bulbophyllum hamelinii* is the giant of the genus with leaves almost 40 cm long and a large,

malodorous inflorescence. Polystachyas can be found in the lower strata of the forest and also on the ground; there are over 20 different species on the island. It is often difficult to identify them because the flowers may look superficially similar but are variable in colour and size. *Polystachya clareae* with its bright orange flowers is readily identified. A fair number of *Liparis* species can be found in leaf litter on the forest floor, a distinctive one being *Liparis longicaulis* with a long rectangular stem and flowers that can be up to 3cm. *Gastrorchis* are amongst the most colourful terrestrial orchids in the area: *Gastrorchis tuberculosa* being one of the most striking; *G. pulchra* var. *perrieri* seems to thrive in cultivation (*G. steinhardtiana* is a synonym of it).

One of the highlights of the Madagascar orchid flora is *Cynorkis*, of which there are about 120 different species, most of them terrestrial but several species can be found growing in moss as true epiphytes like the tiny *C. peyrotii*. Others grow lithophytically, one of the finest examples being *C. lowiana* with very large flowers, which grows on dripping rocks in deep shade. *Physoceras* is very close to *Cynorkis*, the main difference is that the leaf is carried on a long stem. *Physoceras violaceum* grows in thick moss at the base of trees or on lower branches.

Central highlands and mountains

Although there is some overlap with the eastern habitats, the central plateau, including a number of mountain ranges, covers nearly 40% of the island. The climate is more seasonal than the east, with an annual rainfall between 1200 and 2500mm, and there are frequent mists in some areas. Mean temperature in the coldest months ranges from over 13°C in the East and West down to 5°C in high mountain zones. The remaining forest is very much fragmented and mainly composed of moist montane forest. The tree canopy is up to 25m with low-branched trees bearing numerous epiphytes, and there is a thick herbaceous understory. At higher elevations or on exposed ridges there are formations of sclerophyllous montane vegetation, also known as lichen forest, with smaller trees, again many epiphytes, but the understory is quite open and the soil is covered with moss and lichen. At lower altitude the principal vegetation consists of ericoid thicket, with a dwarf open habit generally just a few metres in height. These areas of open woodland are sometimes dominated by Tapia, *Uapaca bojeri*. The trees have thick, fire-resistant bark allowing them to survive the fires which almost annually burn the coarse grassland around them. Tapia belongs in the family Euphorbiaceae, the fruit being edible. The tree is a host of the Malagasy silkworm, from which beautiful textiles are woven.

There is a very different ecosystem towards the summit of the mountains. Frosts and blizzards have been reported during the early morning hours of the dry season, a period lasting approximately seven to eight months, but mist and dew are common. These areas are recognised as containing many endemic plants with highly restricted distribution. Many of the massifs are not protected, although they contain numerous locally endemic species. The region is spotted with inselbergs (isolated monoliths of smooth rounded granite) in the central area and these have similar characteristics. Most of the region, however, consists of a complex blend of hills and valleys, covered by vast expanses of depauperate grassland or pseudosteppe of a few cosmopolitan fire-adapted grasses. There is evidence that some of this grassland was once thinly wooded. Apart from vast tracts of introduced trees like Pine and *Eucalyptus*, these are also the main areas of human habitation and food production. The region rests on degraded laterite soils. Annual man-induced fires are used to produce new herbaceous growth for grazing. In localities protected from fire some forest survives, particularly in gulleys. The orchids of these areas are now becoming quite scarce, most relying on specific microhabitats to thrive. They include many of the species that are seen in cultivation in Europe and elsewhere; it is the region closest to the capital Antananarivo and so it has been more heavily collected. Typical orchids from the region are: *Angraecum magdalenae,* growing in small groups on rocky outcrops, often sheltered by boulders or rock faces. The plants have slightly succulent leaves, large, fleshy flowers emerge on a short stem from beneath the leaf. *Angraecum sororium*, also from mountainous ground and tapia forest can grow quite tall, up to one metre, with elongated thin leaves. Smaller angraecums found in lichen forest or near the summit of some of the mountains are *Angraecum popowii* and *A. rutenbergianum*, both

bearing relatively large flowers. From moister and more shaded forest comes the monotypic *Lemurorchis madagascariensis.*

In many ways the terrestrial orchids of this area are even more exciting and interesting. *Cynorkis* are very well represented with some large and colourful species: *C. gibbosa* can occasionally be seen on the roadside and is not uncommon in the foothills of some of the mountain ranges. The leaves have characteristic darker patches and can be 60cm long, they develop over a very short period, the plant flowers and the leaves then die back. Another locally common species found near cultivated land is *Eulophia plantaginea* , growing along streams and even reported in drainage ditches around rice paddies. Two of the most colourful terrestrials are the blue *Disa buchenaviana* which grows in fairly dry grassland and is locally common and *D. incarnata* grows in the same area but is always seen in boggy ground, it often grows together with *Satyrium trinerve*.

Dry areas of the south & west

The land gradually slopes westwards from the Central Highlands to the coast with remaining forest extending in a broad zone towards the coast from the northern tip of the island. The western zone receives rainfall only during the wet season with a dramatic reduction from the north to the south, ending in areas that receive less than 400mm of precipitation per year. The area supports a variety of sclerophyllous deciduous woodland, open woodland and wooded savannah plus small patches of humid vegetation along rivers and in *Raphia* marshes. The main primary vegetation consists of western deciduous forest which is hot and dry. Rainfall averages 500-1500mm and there is a long dry season of 5 to 10 months, having a profound effect on the vegetation.

The deciduous forest changes towards the south into spiny forest. Here the canopy is 10 to 15 m tall. The extreme south and south-west have an average rainfall of 700mm or less, the driest parts not even getting 300 mm. The climate is arid and hot, the average annual maximum temperature is 30 to 33°C, the minimum 15-21°C. Many plants have adaptations to store what little moisture is available and to minimise water loss. Vegetation is mainly low growing. This xerophytic forest, also known as spiny forest or bush, is dominated by *Euphorbia* and Didieraceae, the world's greatest diversity of baobabs and many other endemic succulents occur here. It is interspersed with limestone formations with their own unique vegetation. The northern part of the island is characterised by a wide variety of topography, with high mountain peaks and coastal plains. Its climate is similar to that of the east except for a much drier pocket at the northern tip.

Orchids in these habitats are not plentiful but they make up for this by their variety of form and interesting adaptation to extreme conditions. A species like *Grammangis spectabilis* from the Zombitsi forest in the south-west is a smaller and modified form of *G. ellisii* which is widespread on the eastern side of the island. *Paralophia epiphytica* has an interesting history; fragments of evidence of the existence of this unusual epiphyte go back to the 1970s. The horticulturalist Marcel Lecoufle found two epiphytes with *Eulophia*-like flowers growing on palm trees in the vicinity of Tôlanaro. One was identified as *Eulophia palmicola*, which had been described by the Henri Perrier de la Bâthie in 1935. The other was left with Jean Bosser at the Muséum in Paris who recognised it as a possible new species, but unfortunately insufficient herbarium material was available and he decided to await further evidence. In 1994 we found large colonies of the beautifully scented epiphyte in full flower in cultivated oil palms next to a swampy area of *Raphia* palms near Fort Dauphin, early the next year a group of botanists from the Royal Botanic Gardens, Kew visited the same site, herbarium and a living collections were made and the latter flowered at Kew in May 2001. With good herbarium material available Cribb *et. al.* then described it as *Eulophia epiphytica*. Based on molecular study it was recently placed in its own genus and is now known as *Paralophia epiphytica* (Cribb & Hermans, 2005). The host trees and the orchid have now almost completely disappeared; only few very small colonies remain.

The genus *Sobennikoffia* is restricted to warm and dry areas. Little difference can be found between the two main species: *S. robusta* has different leaves and a longer spur than *S. humbertiana*. The ultimate in succulence comes in the leafless *Vanilla*. *Vanilla perrieri*, with its impressive bright yellow flowers, is commonest in coastal scrubland, or littoral forest.

The dry soil of this area provides the habitat for a variety of interesting terrestrials. The genus *Oeceoclades* is mainly found here and shows a fascinating array of shape and colour of plant: species like *Oeceoclades spathulifera*, *O. gracillima* (now incorporating *O. roseovariegata*) and *O. boinensis* have not just interesting flowers but also very intricately patterned leaves.

Orchid Mapping

We are involved in an interesting project at the Royal Botanic Gardens Kew to reassess the vegetation of Madagascar. Up-to-date maps of surviving primary and secondary vegetation have been compiled using satellite imagery and ground-truthing. The resulting maps have provided the Madagascan Government with a template for the erection of new reserves to ensure that all the distinctive habitats of the island are protected. Previously, less than 50% were covered in Nature Reserves and other protected sites, mainly because the data on which they were based came from zoological sources. In particular, some distinct vegetation types, such as those found on high mountains, tapia forests, inselbergs and other specialised areas have been greatly reduced in extent and were not included in any of the current national parks or protected areas.

We have also been able to assess the distribution and vulnerability of some Madagascan orchid species. Distribution maps have been constructed, mainly based on data from herbarium sheets and other vouchered specimens with the data collected from the larger European and Madagascan herbaria and including historic as well as more recent records. The distribution patterns are being plotted onto maps that distinguish the remaining vegetation on the island based upon details of aerial mapping, geology and elevation (Du Puy & Moat in press). These maps can be used to indicate where species occurred historically, whether the vegetation they once occupied still persists and where a species might be found in surviving stands of natural vegetation. The maps confirm that some species are indeed endangered but also that others survive in several localities although often in very restricted habitats.

Some species have a limited geographical range, like *Eulophiella elisabethae* which is only found on the Masoala peninsula in the north-east. The orchid favours the palm *Dypsis fibrosa* as a host.

Eulophiella roempleriana is restricted to a narrow ecological range, only being found in the crown of tall screw-pines, *Pandanus*, which in turn grow in swampy ground in forests. Its distribution is confined to the eastern forest from Ranomafana in the south-east to Isle aux Nattes in the north-east, where the few remaining plants are used as a tourist attraction. In contrast, *Angraecum longicalcar*, *A. magdalenae* and *A. henrici* are more restricted in their distribution, being found on the central plateau in confined habitats. The results (Cribb *et al.*, in press) also suggest that species from the eastern rain forest are currently in decline and that those from the central plateau probably declined many years ago when the central plateau was cleared. *Angraecum sesquipedale* is widely distributed throughout the eastern part of the island but it is most common near the coast. Towards the south there are small scattered populations in littoral forest. Here they grow terrestrially in white sand that is now being mined for titanium dioxide. Some of the populations, but by no means all, from the dry south have narrower leaves and a single-flowered inflorescence, this variant was described as *A. sesquipedale* var. *angustifolium* but is still seen in cultivation under its old name of *A. bosseri*.

Another interesting observation was made regarding *Aerangis ellisii*, which rather puzzlingly was recently put on Appendix 1 of CITES, together with, for some reason, two of its synonyms. We all know that the species is fairly common in cultivation from seed-grown stock and there is no shortage of plants in nurseries. When looking at its distribution it is clear that the species is widespread, growing epiphytically in the eastern forest and rock on higher ground. In many ways it has a relatively good chance to escape extinction or even endangerment.

Threatened Plant Appeal

Kew, along with its partner institutions, is committed to an integrated approach to plant conservation that combines in situ and ex situ methods. In other words, it is useless protecting species if their habitats are disappearing. We need to conserve the habitats so that, if plants become threatened, remedial action can be taken such as better management of the environ-

ment or even reintroduction of endangered species.

Notwithstanding the commitment by the new Madagascar government to its natural resources, there is little doubt that much of the island's unique flora and fauna are under threat. The main encroachment comes from agriculture and fires running out of control from grass burning to stimulate new grass growth. These fires are almost essential for the survival of the large herds of cattle which are so important to Malagasy culture. Other threats are from logging, gathering for fuel, mining and to some extent collecting of desirable species, a few orchids are also harvested for medicinal purposes like *Vanilla madagascariensis* and some *Eulophia* & *Cynorkis* species. There is surprisingly little information available on the conservation status of the orchids on the island, as a consequence there has been little systematic conservation planning. Several major projects, notably by the Royal Botanic Gardens Kew in collaboration with Parc Tzimbazaza and other local and international partners, are trying to address this.

One of the major initiatives is the Madagascar Threatened Plant appeal organised through the Friends of Kew which has not only raised a considerable amount of money but also created a greater awareness of the challenges Madagascar faces. The aim is to produce an integrated approach to plant conservation, utilising a number of horticulturally interesting orchids, palms and succulents as flagship species. The six target orchids are: *Aeranthes henrici*, *Angraecum longicalcar, A. magdalenae, Bulbophyllum hamelinii, Eulophiella roempleriana* and *Grammangis spectabilis*. Funds have been spent on conservation assessments and research on distribution. The Centre Technique Horticole d'Antananarivo has been able to purchase materials and equipment for micro-propagation of the target species, a new orchid house at the Parc Tzimbazaza near Antananarivo has been constructed and others renovated for ex-situ conservation. Training has been provided and extensive fieldwork by Kew and local botanists has been undertaken. Work has concentrated on assessing conservation status, distribution and the study of population structure and pollination biology. A long-term aim is to reintroduce selected species to areas from which they have been lost. Efforts by private individuals and nurseries have also contributed to the wider availability of seed-grown orchids. Surplus seedlings will be sold through the trade to finance further work. Educational material in the form of posters and displays have been produced in three languages: Malagasy, French and English.

Orchids as environmental indicators

A checklist and bibliography of the orchids of Madagascar was published more than 5 years ago (Du Puy *et al.*, 1999), the book has now sold out. The work sparked a renewed interest in the orchids of Madagascar and surrounding islands; important fieldwork was undertaken by several individuals and organisations. This, together with the continued work by the botanists working at the Herbier National in Paris, especially Jean Bosser, has meant that a considerable amount of additional information has become available. Several genera have been revised and new species described.

Madagascan Orchids, a revised version of the Checklist and Bibliography (Hermans *et al.* 2007), will be published shortly and this will be another step towards a full revision of the orchid flora of the region. In the new book, the checklist has been updated, idiosyncrasies in nomenclature have been resolved, much additional information on distribution, habitat and flowering time has been extracted from herbarium collections and incorporated and a number of oversights and errors have been amended. Almost one hundred changes in nomenclature are proposed in the new book A large number of species are illustrated. Further details can be obtained from j.harris@rbgkew.org.uk.

Orchids are found in almost every habitat in Madagascar and are ideal plants for use as indicators of environmental health. We have a good idea of where orchids were historically distributed, field work is providing a clear idea of modern distributions and threats. An illustrated *Field Guide to Madagascan Orchids* is in an advanced state of preparation that will allow plants to be named in the field. Distributions affected by environmental changes can then be rapidly monitored. The orchid data can then be assessed alongside data from birds, butterflies, lemurs, amphibians and reptiles to provide a clearer idea of the health and conservation needs of any particular habitat.

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LITERATURE CITED

Cribb, P., D. Roberts & J. Hermans. 2005. Distribution, ecology and threat to selected Madagascan Orchids. In: Selbyana. Proceedings IOCC II. In press.

Du Puy, D., P. Cribb, J. Bosser, J. & C. Hermans. 1999. The orchids of Madagascar, annotated checklist and annotated bibliography. Royal Botanic Gardens, Kew. 424 p.

Du Puy D. & J. Moat. 2003. Using geological substrate to identify and map primary vegetation types in Madagascar. Pp. 51-67 *in*: S. Goodman & J. Benstead. 2003. The Natural History of Madagascar. University of Chicago Press, Chicago & London.

Goodman, S. & J. Benstead. 2003. The Natural History of Madagascar, University of Chicago Press, Chicago & London. 1709 p.

Hermans J. & C., P. Cribb, J. Bosser & D. Du Puy.2007. Madagascan Orchids. Royal Botanic Gardens, Kew.

Moat, J. 2005. Vegetation map of Madagascar. At www.vegmad.org.

Dr **Phillip Cribb** studied at Cambridge and Birmingham in England before, in 1974, joining the staff of the Royal Botanic Gardens, Kew, where he was Deputy Keeper of the Herbarium and Curator of the Orchid Herbarium until his retirement in March 2006. He currently holds Honorary Fellowships at Kew and at the Royal Holloway College, University of London. He has participated in many expeditions to study plants in the field, especially in the tropics of Africa, Madagascar, SE Asia, the Americas and the Pacific. He is the author or co-author of over 360 scientific papers and books on orchids. His current research is concentrated on a new classification and evolution of the family Orchidaceae, and on the taxonomy and conservation of orchids. He was Chair of IUCN (The World Conservation Union)/Species Survival Commission's Orchid Specialist Group from 1994 until 2005.

Johan Hermans has travelled extensively in Madagascar. He is, by profession a conservator and is currently head of the conservation department at the Museum of London. He is the author of many articles on orchids and co-author of *Orchids of Madagascar* (1999) and *Angraecoid Orchids* (2006). He is currently an Honorary Research Associate at the Royal Botanic Gardens, Kew, chairman of the Royal Horticultural Society's Orchid Committee and of it Advisory Panel on Orchid Registration. He is also a keen orchid grower and has specialised in Madagascan orchids.

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COMMUNITY INVOLVEMENT IN ORCHID CONSERVATION

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In 2000 the Australasian Native Orchid Society, Victorian Group Inc. (ANOS Vic) made the decision to become proactive in orchid conservation. It was apparent that government organisations were over stretched when it came to devoting the resources needed to protect our native orchids and that as a society we could assist in many ways. Through working with the authorities responsible for orchid preservation we have been able to make a substantial contribution, in some cases taking a primary role, in the management and recommendations for the preservation of habitats and orchid species. The activities we have been involved with include monitoring, searching, cultivation, propagation, rescue digs, translocation, associated species mapping, reintroduction, environmental management and public education.

Collectively our members have a wide range of skills. Many members are skilled in orchid identification in the field, others have developed cultivation skills from many years experience and many have brought skills from their various professional backgrounds. These skills can be used to assist in identifying associated and invasive plant species, population mapping and monitoring, and promoting public awareness, amongst others.

Many members have retired and can be called upon to assist at short notice. As a volunteer group that is dedicated to native orchids, we provide continuity to a project that is not necessarily present in official organisations. Employees can leave to find other employment or be transferred to other projects as the need arises, whereas people that undertake this work as a passionate hobby tend to actively participate for many years. This paper looks at the various projects and activities that ANOS Vic. members have participated in.

Monitoring

Monitoring orchid populations in the field can be an invaluable asset to understanding the life cycles of endangered orchids over extended periods of time. Without this information it is difficult to assess whether an orchid population is truly endangered or is just being influenced by current meteorological conditions. It is work that needs to be conducted regularly and depending upon the species, several times of year at critical stages of the plants development. ANOS Vic has been regularly monitoring several orchid species throughout Victoria.

We have been responsible for monitoring *Pterostylis despectans* (Lowly Greenhood) since 2000, an endangered terrestrial orchid that grows in the goldfields of Victoria. Monitoring is undertaken twice a year, followed up by seed collection. The first monitoring is undertaken in late July when the rosettes have emerged. This is the best time to find the orchids as the leaves can be readily identified, whereas later in the year when the orchids are in flower the leaves die back and the flower tends to blend in with the surrounding ground. We have established three monitoring sites where we count all rosettes, pinpoint their location and measure rosette size and number of leaves. We also visit all known sites within the state and count all the rosettes we can find. This is something that requires a large group of people who are skilled in identifying the orchids from the rosettes. In December we return to the three monitoring sites to record the progress of the rosettes to see if they have flowered, withered away or have been dug up by White Winged Choughs, a native bird that regards these orchids as a delicacy. We also undertake hand pollination of the flowers as natural pollination rates are low. The seed is then collected and preserved in storage or used to help establish ex-situ populations.

During the period of our monitoring the area has been impacted by an extended drought. This has resulted in an average decline at our three monitoring sites at an average of 12% per annum. It is essential that we continue this monitoring through this period so that we are able to record what the true impact of the drought has been on the orchids when it eventually breaks.

Since undertaking the responsibility of monitoring *Pterostylis despectans* we have since been involved in other monitoring programs. Our monitoring programs now include *Pterostylis woollsii* (Long-tail Greenhood), *Pterostylis basaltica* (Basalt Rustyhood), *Pterostylis maxima* (Large Rustyhood), *Pterostylis cucullata* (Leafy

Greenhood), *Prasophyllum suaveolens* (Fragrant Leek-orchid), *Diuris fragrantissima* (Sunshine Diuris), *Diuris basaltica* (Small Golden Moths) and *Diuris punctata* (Purple Diuris), all orchids that are classified as endangered or vulnerable and a list which grows each year.

Searching

This is another activity that is well suited to large groups of volunteers that know what they are looking for. We tend to concentrate on orchids that are classified as endangered. Most of these orchids are known to exist at only one or two locations, but in many cases no concerted effort has been made to verify this, principally because of the large areas involved and the amount of time it takes to conduct searches. ANOS Vic now conducts regular annual searches, targeting particular species. Our searches are based upon previous records of unconfirmed sightings, as well as searching the state Flora Information System (FIS) for areas similar to the currently known sites. To improve our results we have also undertaken associated species surveys at known sites and fed this information into the FIS to help refine our searches. This technique has had very encouraging results. When we first started monitoring and searching for *Pterostylis despectans* the population was estimated to be around 500 plants based upon four known colonies within a 24 km area. We have now discovered around 40 sites over a 70 km area that contains over 4,500 plants.

The searching technique we employ is to visit the regions that the FIS indicates a match to the vegetation profile. We then drive through the regions looking for areas that look "right" as compared with our known sites. We used to then sweep through an area in a line undertaking a thorough search. This approach worked to some extent, but the line gradually broke down as people wanted to follow their instincts. Now we break the main group up into smaller groups of between 2 and 4 people each, making sure that each group has a hand held GPS unit so they do not get lost and so they can record the position of any findings. This then allows the groups to follow their instincts and search in spots which they believe to be favourable conditions for the particular orchid. This seems to be more productive and more satisfying for the searchers.

Often searches are undertaken at the leaf or bud stage. This may not lead to positive identification of the particular species required but it does mean that we generally find more plants as some may wither and die back before flowering, depending upon the amount of rainfall received. Any potential plants are recorded on GPS and sometimes caged if we are concerned about the plants being grazed. A couple of people can then return at the peak flowering time to positively identify the orchid and record its status.

Another very successful search was conducted in September 2006. Conservation officers from the Department of Sustainability and Environment were aware of a small colony of 16 *Diuris basaltica*. ANOS Vic were called upon to assist them with the searching and monitoring for this orchid. Despite a very dry season our members were able to discover over 500 plants by undertaking a thorough search of the area, a very satisfying result for all involved.

Propagating

ANOS Vic members have built up many skills over the years in native orchid cultivation. This has required careful study of the plants requirements and the development of a potting mix suitable for terrestrial native orchids in cultivation. The society has also published its own book the "Cultivation of Australian Native Orchids". This book has been written by members and all proceeds from the book go back into the society to help finance our activities and conservation work.

There has been a great deal of study undertaken in the propagation of epiphyte orchids from seed, but much less work in the cultivation of Australian native terrestrial orchids from seed. In 1999 a seed propagation group was formed to develop techniques suitable for terrestrial orchid propagation. The Australian Orchid Foundation donated a lamina flow cabinet to the society and one of our members, Dick Thomson, turned one of the rooms in his house into a propagating laboratory that members could use. This laboratory has recently been expanded to two lamina flow cabinets as the demand by members to use the facility has grown.

After developing our techniques on orchids that are reasonably easy to grow we have since expanded to try to propagate orchids that are endangered and have not been grown in cultivation before. By obtaining seed collection permits we have been able to experiment with growing endangered species with the aim of establishing ex-situ populations that can later be used to produce seed for sowing in the wild or to produce plants to return to the wild.

Our efforts have had mixed success. One particular success story has been with *Diuris fragrantissima*. This orchid was quite common on the grassland plains west of Melbourne. Because of loss of habitat, due to urban development, the orchid has become highly endangered with only about 20 plants now known to exist in the wild. This orchid was adopted by Melbourne Zoo, which has a conservation charter for plants as well as animals. The orchid was grown from seed and dedicated shade houses were built to house the plants. As it turned out these orchids were relatively easy to grow from seed and grew

well in cultivation. ANOS Vic members regularly assist the Zoo when it comes to deflasking and potting up the seedlings. Our members are also growing the orchid in their own shade houses so that the entire collection is not held at one location, as this could prove fatal if a virus was to establish itself in the only ex-situ population.

After several years of building up this collection, plants are now being reintroduced to the wild at reserved sites. Our members have assisted with the planting and now members that live close to the site keep a regular eye on them and also assist with watering the orchids while they are becoming established. About 700 plants have now been reintroduced and between 60% and 70% of them emerged in 2006.

We have also experimented with collecting orchid material from damaged orchids and planting it to establish new plants. The *Pterostylis despectans* we have been monitoring come under regular attack from White-Winged Choughs. These birds dig down, pull the plant out of the ground, eat the tuber and leave the remainder of the plant to die on the surface. When we conduct our monitoring we collect these scraps and plant them in our native orchid potting mix. Quite often the plant material will send out a root which will go on to form a tuber which becomes part of our ex-situ population.

Environmental Management

Having a membership of around 450 gives us a substantial work force. We have undertaken many weeding activities at various sites ranging from confined areas to much larger sites. Some sites require one or two visits to eradicate particular weeds, while others require ongoing annual visits to remove seedlings germinating from seed that can stay viable in the soil for many years.

One project that we started in 2000 was with *Prasophyllum suaveolens*, growing in a country cemetery in Western Victoria. When this orchid was brought to our attention only 39 plants could be found and the area where it grows was overgrown with *Watsonia* and surrounded by Broom seedlings. The area was also being mown every spring just as the plants were coming into flower, so no new seed was being dispersed. In conjunction with the Cemetery Trust who were not aware of the presence of the orchid, the Department Sustainability and Environment which had classified the cemetery as a remnant of native grasslands and the local municipal council who were responsible for mowing the cemetery a management plan was established to protect the orchid and yet still enable the cemetery to operate.

ANOS Vic established a monitoring program to record all the orchids. We then conducted a complete topographic survey of the entire site, plotting the position of the orchids with respect to the graves. This enabled us to draw up a plan of areas safe to mow which also allowed access to all the graves. Since establishing the project three controlled burns have been conducted. These were conducted in early autumn and were to promote orchid growth and at the same time weaken the Watsonia and Broom. ANOS Vic members then followed up with hand weeding of the Broom as the fire causes the seed to germinate. We also hand paint the Watsonia with herbicide as it emerges. This program has had substantial results. The number of recorded orchids is now at 277. Unfortunately not everything goes to plan and to some extent our efforts have had too great an effect. By the end of 2004 the native grasses were thriving, becoming the dominant species. It was intended to conduct a controlled burn in early April 2005. Unfortunately it was considered too dry and therefore too dangerous to conduct the burn considering the amount of combustible material. By the spring of 2005 when we conduct the monitoring, the grasses were so long and thick that it was impossible to locate the orchids without risking potential damage because of trampling. In April 2006 it was considered safe to conduct a burn. This was followed up by a period of severe drought. The orchids that emerged during the winter then all aborted and died back in the spring, leaving nothing to monitor. However on the good side the Broom seedlings that germinated after the fire all died due to lack of water and the remaining *Watsonia* had been substantially weakened.

Apart from weed work which has been conducted at many sites we have also been actively lobbying to have areas fenced off to protect special orchid areas. Fencing, as well as our other activities, requires funding. Since 2000 the society has raised over $42,000 for orchid conservation through grant applications and from community and members donations. Part of this funding has enabled us to get two orchid rich areas fenced off from 4WD vehicles and motor cycles that were damaging the environment.

Orchid Rescue, Translocation and Reintroduction

Having established our credentials with various government organisations we find that we are now being called in for advice and assistance in rescuing and translocating orchids.

One example has been with Vic Roads who called us in to assist with translocating some *Diuris chryseopsis* (Golden Moths) that were in the path of a new freeway. These orchids could not be just dug up and replanted as there was no new site available for them to be translocated to. The plants had to be removed, kept alive for two years while the new site was purchased and prepared and then translocated. As our members have considerable experience in native orchid cultivation our society was

the natural choice. To assist with this project Vic Roads donated a portable shade house to the society that could be erected on a member's property to allow the orchids to be cared for. The plants were then dug up and placed in foam boxes with as much of the original soil around them as possible. Two years later when the new site was ready the orchids were in a very healthy state to be returned to the wild. As part of this project local residents were actively involved with the removal and planting, they then took over the responsibility of keeping an eye on the newly planted orchids and watering if necessary to assist them with re-establishment.

A similar project has been undertaken with *Pterostylis cucullata* which grows on the Mornington Peninsula. This is another case of an orchid loosing its habitat due to development. On a private block of land in the area, the owners applied for a permit to build on it. It turned out that *Pterostylis cucullata* was growing on the site and one of our local members was called in by the municipal council and Parks Victoria to remove the orchids from the site. Over 2000 orchids were collected. These plants had to be maintained for one year while Parks Victoria selected a suitable site for translocation. The tubers were then introduced to the new site in early 2006 while they were dormant and approximately 70% emerged the following spring.

Public Education

Because the *Pterostylis cucullata* is growing in amongst residential development the council decided that it would try and map the extent of the populations. One of our members conducted door knocks to explain to residents about the significance of the orchid, how they could identify it and how they could manage it if it was on their land. The residents were also asked for permission to enter their properties and conduct a search to see if the orchid was growing there. In most cases the response was positive and through the use of hand held GPS a map was able to be built up of the colonies in the area. This has given the council a better idea of how to manage future development within the area.

ANOS Vic holds an orchid show each year and also attends other shows where we can meet the public. At these shows we now focus on conservation to a much greater extent. The orchid display draws the general public in and then gives us the opportunity to talk to them about conservation and the plight of many of our orchids. We now find that many of our new members are joining primarily to actively participate in conservation rather than just to go out into the bush to look at orchids, or learn how to grow the best flowers.

Prime Minister's Banksia Award for the Environment

Each year the Prime Minister of Australia presents the Banksia Awards for the Environment. There are several categories in these awards and because of the large amount of successful work done for orchid conservation by several organisations working in collaboration, it was decided that a collective submission should be made in 2006. The submission, in the category of Land and Biodiversity, was made on behalf of the Department of Sustainability and Environment, Parks Victoria, Royal Botanic Gardens, Melbourne, Melbourne Zoo, Melbourne University, Victoria University, Royal Melbourne Institute of Technology University and of course the Australasian Native Orchid Society, Victorian Group. This is Australia's most prestigious awards for the environment and you can only imagine our pride in our achievements when renowned primatologist Dr. Jane Goodall announced that we had won. We certainly do not undertake this work for the awards, we do it for our love of orchids, but it is satisfying to get recognition for the work that been undertaken.

Conclusion

This paper has set out to demonstrate practical cases where community organisations can make an effective contribution to orchid conservation. In today's world, discussions about the environment are on everyone's lips and more and more people want to make a practical contribution to preserving our world. Individuals can't change everything, but if orchids are your passion then concentrate on what you love. I hope to inspire other individuals and community groups that have a love for orchids, to follow our example and to encourage official organisations to tap in to this valuable resource.

Andrew Dilley is the President of the Australasian Native Orchid Society (Victorian Group) Inc. From 2000 to 2005 Andrew was the society Conservation Officer where he initiated a proactive conservation program and with the help of the society's members, has watched it grow to an extremely enthusiastic active group. Andrew enjoys growing terrestrial orchids and experimenting with seed propagation. He loves being in the field looking for orchids and is an avid photographer. Andrew's academic background is in land surveying, which comes in handy when it comes to monitoring orchid populations. When he isn't working with orchids Andrew is a Director of Listech Pty. Ltd. a company which develops software for the surveying, mapping and civil engineering industries.

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INTEGRATED APPROACHES TO ORCHID CONSERVATION IN GUATEMALA: PAST, PRESENT AND FUTURE, OPPORTUNITIES AND CHALLENGES

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RESUMEN. Se discuten los retos enfrentados para lograr la conservación de orquídeas en Guatemala, los factores que contribuyen a la pérdida de poblaciones, y los esfuerzos realizados para preservar las especies. Se presentan ejemplos de estrategias que combinan técnicas *in situ* y *ex situ* e incluyen aspectos educativos, mitigación de impactos ambientales, rescate y participación comunitaria.

KEY WORDS: orchid conservation, Guatemala, rescue, community participation

Introduction

In 2001, Dix *et al* described the state of orchid conservation in Guatemala, factors responsible for orchid disappearance, legal aspects that favored or hindered conservation efforts and the relative success of different approaches. We present an update on the Guatemalan scene and discuss some indications that give hope for the future.

Factors that contribute to orchid loss in Guatemala in order of importance are: land use change that includes deforestation for agriculture, timber production, conversion of mixed forests to conifer plantations, and losses from forest fires; cloud cover reduction as a result of climatic change; disappearance of pollinators, especially bees; collectors and street vendors; and herbicide use that affects both pollinators and terrestrial orchid populations. The latter are particularly vulnerable, represent 30% of the Guatemalan orchid flora (Dix & Dix 2006) and many have disappeared from habitats in and around Guatemala City where they were once abundant. In rural areas, terrestrials are threatened by leaf litter and humus removal for horticulture, as well as destruction of the litter layer by exotic worms (Dunne, 2004). Closely related to these factors is the extreme poverty of many communities in regions where orchids are, or were, abundant.

Approaches to Orchid Conservation

These include *in situ* habitat preservation, either in national or private reserves; rescue operations with either *in situ* or *ex situ* orchid care; restoration and management; seed bank development and seedling propagation; and education. Many of these tactics can be combined to form an integrated strategy.

RESCUE OPERATIONS. Given that prevention of habitat loss is often not feasible, rescue operations are potentially very important. Tree cutting, for lumber or slash and burn agriculture, results in a large amount of orchid material which could be rescued, incorporated into tourist attractions or used to establish propagation sites that could generate income for small farmers, especially those living in the multiple use zones of protected areas. In addition, because of destruction by fire, Guatemala annually loses hundreds of millions of orchid plants, representing millions of dollars.

In the past, rescue activities were hindered by government policies that required not only constant inventories but also prohibited vegetative reproduction of rescued plants for commercial purposes. This situation may have changed. It seems that the authorities in CONAP (National Council for Protected Areas) charged with managing and protecting biodiversity in Guatemala, have realized that without at least an opportunity to cover costs, there is little incentive for raising and caring for rescued or confiscated plants. Now, CONAP allows rescue operations by registered nurseries and collections and permission can be given to collect in areas scheduled for urban development, use conversion (*e.g.* conversion of citric orchards to sugar cane plantations) and forestry operations; provided one has the owner's permission. Moreover, these plants can be used for vegetative propagation and divisions sold. In the past, one was required to wait for the third generation.

COMMUNITY INVOLVEMENT. Because there was usually no local community involvement in rescue operations, no economic benefits were perceived and projects perished from lack of funding. At the Universidad del Valle, we are currently developing a program with community involvement that we hope will eventually contribute to sustainable orchid use. This project, financed by the U. S. Food for Peace program, is being carried out in collaboration with Texas A & M University (TAMU) and the funds received from selling surplus soy flour in Guatemala are used to promote income generating, horticulture projects in local communities. Our project involves developing central nurseries for orchid and bromeliad propagation in the university's two rural campuses, located in the highlands above Lake Atitlan at 2,300m and on the Pacific coastal plain at 300m, and developing a cooperative that will work with satellite nurseries in five surrounding communities in each region. The central nurseries will be the plant source for community nurseries. Stock plants will be obtained both from rescue operations and from local and foreign purchases. Initially, only a few selected species, suitable for the different climatic regimes will be commercialized in these operations; later, plants grown from seed will be incorporated into the system. Genera with species that we hope to conserve in this way include *Guarianthe*, *Rossioglossum* and *Lycaste.* Once this is established, we hope to be able to repopulate community forests.

At the same time, plans are underway to develop a seed bank at the central campus. The university also has an established field station of over 1,000 hectares with at least 120 orchid species on Volcán Atitlán and a reforested ravine on the central campus with many terrestrial species.

MINES AND RESCUE OPERATIONS. In Guatemala, at present, mining development, as a government policy, has generated public opposition. Nevertheless, at least two of the mining operations have proposed establishing nurseries for cultivating epiphytes that they rescue from felled trees as part their management and impact mitigation plans and intend to transfer plants to reserve areas within the mining operation. One of these has set up the nurseries but, as yet, does not know what to do with the plants they are raising. The other, which has not yet begun operations, plans to rely more on transferring orchids and bromeliads to refuge areas that have been set aside within the mining operation.

PROTECTED AREAS. Thirty one percent of Guatemala's territory is included in the national protected area system (SIGAP), but these reserves are not always managed adequately because financial resources are insufficient (CONAP 2006). During the past few years, a series of private reserves, many of them located in cloud forest remnants, has gained momentum and could potentially result in better orchid protection, income from tourists and educational programs. A recent undertaking that gives fiscal incentives to communities and landowners for maintaining existing forest cover is a positive development. Yet, the areas where most orchid diversity is found are also those with most deforestation.

EDUCATION. Education can inform as to what orchids are, why they are important, how they are threatened and what could be done to help preserve them. This can have both positive and negative consequences. In Guatemala, because of rural poverty, any potential income source is exploited without thought to its sustainability. Some orchid education programs have resulted in increased depredation, even though the importance of conserving disappearing species was stressed and the participants agreed that orchids were threatened. Another approach that gave talks to street vendors, resulted in attempts to cultivate rather than just sell wild collected plants. However, large numbers are still being collected from nearby forests and these are not rescue operations.

Another Guatemalan has been energetically educating orchid growers, not only in Guatemala but also in El Salvador. His courses have included members of orchid societies, and agriculture and biology students, as well as the sons of one of the largest vendors of wild collected plants. As a result, there is more appreciation of the plight of orchids, and an interest in cultivation and propagation. We hope this will result in a genuine interest in conservation.

A local secondary school, guided by Raquel de Pinto, has worked unceasingly since 1994 (Colegio Ciudad Vieja 2006) to protect and reforest the Colonia Maestra ravine in Guatemala City and has a program where rescued orchids are planted in trees within the Universidad Francisco Marroquin campus. These efforts stimulated the Universidad Francisco Marroquin to develop a uni-

versity arboretum and a website with photographs and species descriptions. Several schools in Guatemala City and two more universities are now carrying out similar projects in nearby barrancos and at least 60 species native to Guatemala City, including 25 terrestrials, are being preserved.

Local orchid societies give occasional talks on orchids and their orchid shows help educate the public, but so far they do not have any established programs for orchid conservation, other than in greenhouses and private collections.

SEED PROPAGATION. This is, as yet, limited in scope. Very few orchidists hybridize or sow seeds of native species. Intensive programs to propagate large numbers of *Lycaste skinneri alba* were abandoned because the institution could not devote personnel to maintaining the flasks. Another program that hoped to sell small seedlings in flasks failed because of insufficient expertise. A long-term commitment and an appropriate financial base is required.

The proposed orchid seed bank may help to establish more *in vitro* orchid propagation. Only a few genetic studies to determine variation and hybrid relationships have been carried out (Maldonado *et al*. 2001).

Concluding remarks

Orchid conservation is a long-term proposition that requires continued effort; local community involvement and perceived benefits are essential. In Guatemala, at least, the people who live closest to the orchid populations cannot afford to be altruistic and, in the long run, projects related to orchid conservation have to be self-sufficient. There is a need for more collaboration and joint efforts involving hobbyists, academics and commercial producers

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LITERATURE CITED

CONAP. 2006. Establecimiento de prioridades nacionales y evaluación de necesidades para la creación de capacidades en biodiversidad, Proyecto GUA/97/G31, fase II. Informe final. Consejo Nacional de Áreas Protegidas. 59p.

Colegio Ciudad Vieja. 2006. Fundamentos para rescatar, proteger y manejar la flora y fauna de la microcuenca del Río Negro en la ciudad de Guatemala. Colegio Ciudad Vieja XXXIX Promoción Bachilleres en Ciencias y Letras. 356pp.

Dix, M.W., M.A. Dix, & M. Maldonado. 2001. Conservation of orchids in Guatemala. Memoria 2o Seminario Mesoamericana de Orquideología y Conservación. p. 17.

Dix, M.A. & M.W. Dix. 2006. Diversity, distribution, ecology and economic importance of Guatemalan orchids. Pp. 187-198 in E. Cano (ed.) Biodiversidad de Guatemala. Volumen 1. Universidad del Valle.

Dunne, N. 2004. Invasive earthworms – a threat to North American forests. Plants and Gardens News 19: 1-4.

Maldonado, M.L., M.A. Dix, M.W. Dix, M. Palmieri & L. Castellanos. 2001. Relaciones genéticas e hibridación natural entre especies de *Lycaste*, Sección Deciduosae, sub-sección Xanthantae (Orch.) en Guatemala. Memoria Segundo Seminario Mesoamericana de Orquídeología. p. 26.

Michael Dix is a researcher and professor in the Center for Agriculture and Forestry Studies and the Department of Biology at the Universidad del Valle de Guatemala. He studied chemistry and biology at George Washington and Harvard universities. He was responsible for establishing the first biology degree program in Guatemala, later founded the Center for Environmental Studies and designed and coordinated the Master's Program in Environmental Studies. Currently, he is developing the community program described here, recently published a revised list of Guatemalan bromeliads and is co-author of a Revised Annotated Checklist of Guatemalan Orchids.

Margaret Dix studied biology at the University of London, and Harvard University. She chaired the Department of Biology for 25 years and co-coordinated the Master's Program in Environmental Studies at the Universidad del Valle de Guatemala. She is a member of the Orchid Specialist Group and the Guatemala Wetlands Commission. Currently, she is involved in developing a monitoring program for mining concessions and is also co-author of a Revised Annotated Checklist of Guatemalan Orchids.

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INVESTIGATION OF PROCESSES LEADING TO THE DECLINE OF SOUTH AUSTRALIA'S *CALADENIA* SPECIES

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Of the 300 species of orchids recorded in South Australia, over one-third have been listed as vulnerable, rare, or endangered (Barker *et al.* 2005). While direct habitat loss is likely to be the major cause for reductions in the former ranges of species, indirect consequences of habitat fragmentation may be responsible for the continued decline of many species within remnant fragments. Reduction of genetic diversity (Ellstrand & Elam 1993, Young *et al*. 1996,), disruption of plant-pollinator interactions (Aizen & Feinsinger 1994, Lennartsson 2002) and changes in habitat characteristics (Tremblay 2005) can all influence the population dynamics and hence, persistence of, a species across a landscape.

We are investigating the potential causes of the continued decline of several orchid species within the genus *Caladenia* occurring within the highly fragmented landscape of the Mt. Lofty ranges. We have made assessments of pollination and reproductive success across a range of habitat fragments and will relate these to the influences of population size, fragment size, isolation, disturbance and local habitat structure. Furthermore, studies over consecutive years will allow us to assess the costs of reproduction and herbivory on future plant fitness. This research provides an insight into processes leading to the decline of some orchid species and hence has important implications for management and conservation strategies.

LITERATURE CITED

Aizen, M.A. & P. Feinsinger. 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. Ecology 75: 330-351.

Barker, W.R., R.M. Barker, J.P. Jessop, J.P. & H.P. Vonow. 2005. Census of South Australian Vascular Plants. 5th Edition. J. Adelaide Botanic Gardens Supplement 1. Botanic Gardens of Adelaide and State Herbarium: Adelaide.

Ellstrand, N.C. & D.R. Elam. 1993. Population Genetic Consequences of Small Population-Size - Implications for Plant Conservation. Ann. Rev. Ecol. Syst. 24 :217-242.

Lennartsson, T. 2002. Extinction thresholds and disrupted plant-pollinator interactions in fragmented plant populations. Ecology 83: 3060-3072.

Tremblay, R.L., J.D. Ackerman, J.K. Zimmerman, J.K. & R.N. Calvo. 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. Biol. J. Linn. Soc. 84: 1-54.

Young, A., T. Boyle & T. Brown. 1996. The population genetic consequences of habitat fragmentation for plants. Tree 11: 413-418.

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ARE SOME LIFE-HISTORY STRATEGIES MORE VULNERABLE TO THE GENETIC CONSEQUENCES OF HABITAT FRAGMENTATION? A CASE STUDY USING SOUTH AUSTRALIAN *CALADENIA* R. BR. (ORCHIDACEAE) SPECIES

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KEY WORDS: habitat fragmentation, life history, population genetics, *Caladenia*, pollination, South Australia

Habitat fragmentation, through land clearing, has been attributed in the demise of many species of plants and animals throughout the world (Kinzig and Harte 2000). Not surprisingly, much research effort has been devoted toward understanding the dynamics of populations subject to fragmentation.

The Mount Lofty Ranges, adjacent to the South Australian capital city of Adelaide, constitute a region where fragmentation, through land clearing, has been prevalent (Paton 2000). The area was historically home to a number of endemic orchid species which are now either extinct or under threat (Barker *et al*. 2005). The contemporary distribution of species that are still present, particularly of the genus *Caladenia* R. Br. (Orchidaceae), is interesting with respect to a diversity of traits in habitat requirements and pollination specificity. Some of these species are quite prolific while others are only found in remnant patches and it is not clear what is driving these differences. It is generally considered that habitat reduction effects plant population dynamics on several fronts by reducing recruitment potential through loss of pollinating agents (Aguilar *et al.* 2006), restricting potential for range expansion (Opdam & Wascher 2004) and interrupting natural disturbance regimes (Coates *et al.* 2006). However, the interactions driving these responses are often complex and management regimes require a thorough understanding of key processes if they are to be successful. In order to evaluate the effects of these variables, this study adopts the conservation genetics paradigm (Ouborg *et al.* 2006) as a means of identifying species and populations that have been effected by fragmentation and aims to associate the level of impact with life history characteristics.

TABLE 1. Pollination specificities (Cingel 2001) for three species of *Caladenia* found in the Mount Lofty region of South Australia.

Pollination Specificity	
High	*Caladenia tentaculata*
Moderate	*Caladenia rigida*
Low	*Caladenia carnea* var. *carnea*

The presenter discussed the results from an investigation of microsatellite allelic diversity and structure among populations of three species expressing a range of pollination specificities (table 1).

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LITERATURE CITED

Aguilar, R., L. Ashworth, L. Galetto & M.A. Aizen. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology Letters 9: 968-980.

Barker, W.R., R.M. Barker, J.P. Jessop, & H.P. Vonow. 2005. Census of South Australian Vascular Plants. 5th Edition. Journal of the Adelaide Botanic. Gardens. Supplement 1 (Botanic Gardens of Adelaide and State Herbarium: Adelaide).

Cingel van der, N.A. 2001. An Atlas of orchid pollination : America, Africa, Asia and Australia. Rotterdam, A.A. Balkema. 296 p.

Coates, F., I.D. Lunt & R.L. Tremblay. 2006. Effects of disturbance on population dynamics of the threatened orchid *Prsophyllum correctum* D.L. Jones and implications for grassland management in south-eastern Australia. Biol. Conserv. 129: 56-69.

Kinzig, A.P., & J. Harte. 2000. Implications of endemics-area relationships for estimates of species extinctions. Ecology 81(12): 3305-3311.

Opdam, P. & D. Wascher. 2004. Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. Biol. Conserv. 117(3): 285-297.

Ouborg, N.J., P. Vergeer & C. Mix. 2006. The rough edges of the conservation genetics paradigm for plants. J. Ecol. 94: 1233-1248.

Paton, D.C. 2000. Disruption of bird-plant pollination systems in southern Australia. Conserv. Biol. 14: 1232-1234.

Lachlan Farrington's research interests are in the application of genetic techniques to the management and conservation of natural systems. He is currently employed as a Research Associate at the University of Adelaide undertaking a study of genetic variation in orchid species found in and around the Mount Lofty Ranges near Adelaide.

José Facelli is an Associate Professor at the University of Adelaide's Environmental Biology department. His interests lie in terrestrial plant ecology, particularly the role of spatial and temporal heterogeneity in the structure and function of ecological systems. Recently, he has initiated several projects studying the impact of fragmented habitats on the genetic diversity and population dynamics of orchids.

Stephen Donnellan uses molecular genetic technologies to investigate issues in natural resource management, conservation biology, biodiversity and systematics. Most of his research has focused on vertebrates from the Australo-papuan region.

Professor **Andy Austin** is the Director of the the Australian Centre for Evolutionary Biology & Biodiversity at The University of Adelaide. His research interests include the systematics and evolution of parasitic wasps, the biology of spiders, phylogeography of short-range edemic species, and the conservation biology of terrestrial and freshwater invertebrates.

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VANDA TRICOLOR LINDL. CONSERVATION IN JAVA, INDONESIA: GENETIC AND GEOGRAPHIC STRUCTURE AND HISTORY

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KEY WORDS: *Vanda tricolor*, haplotype network, nested clade analysis, genetic, geographic, structure

Introduction

Vanda tricolor Lindl. is widespread in cultivation in South East Asia, being relatively easily cultivated in the garden, and is often seen growing floriferously on garden trees, fence posts and verandas. Whilst the species is widespread in cultivation in its native regions of Java and Bali, wild populations are small and highly fragmented. The author's recent field trip to Java and Bali found few wild examples of the species, and the few plants found were in the most inaccessible trees, due to massive over-collection, often with no other *V. tricolor* plants to be found in the area.

A study of *V. tricolor* specimens, collected in the wild by the Indonesian botanic gardens, using several population genetics methods elucidates whether the specimens exhibit any genetic structure and how that might relate to their geographical origins. The results show several interesting points including the putative geographical origin of the species and the dates of the divergence of the species from this origin and into other regions.

Investigation

Thirty four specimens of *V.tricolor* with original collection locality information were collected from all four of the Indonesian Botanic Gardens – Bogor, Cibodas and Purwodadi in Java and Eka Karya in Bali (see Table 1). Leaf specimens were collected for DNA extraction and where plants were in flower, flowers were preserved in spirit for morphological analysis. *Vanda luzonica* Loher ex Rolfe has previously been identified as a putative sister taxon to *V.tricolor* (Gardiner, unpublished data) and was used throughout the genetic study as an outgroup. The *V.luzonica* DNA sequence (referred to as 'VL' throughout) was amplified from DNA sample 19632 in the RBG Kew DNA bank, collected by the author from Motes' Orchids in Florida, USA, and originally from the Philippines.

The original collecting localities of the *V.tricolor* specimens divided into five clear, non-overlapping geographic regions so each specimen was assigned to a geographic region – West Java, Central Java (including D.I. Yogyakarta), East Java, Bali, Central Sulawesi, (Fig. 1). At the national park at Kaliurung on Mount Merapi in Java, there is an effort to cultivate and reintroduce *V. tricolor* to 'protected' areas of the park on a small scale when funds and time are available. The specimens originally collected from 'Central Java' and used in this study are a selection of those being reintroduced.

A portion of the single copy chloroplast DNA region trnL-F was amplified using primers c and f of Taberlet et al (1991), as in Table 2, and sequenced using just primer c. The region amplified includes the 3' end of the 5' trnL (UAA) exon, and most of the trnL intron, and is between 270-319 base pairs in length. During phylogenetic work on the genus

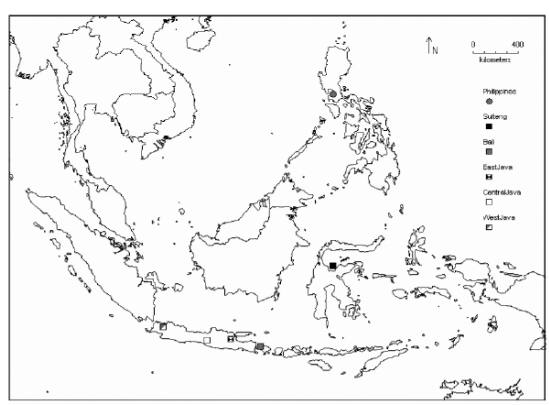

FIGURE 1. Geographic regions from which *V.tricolor* (and *V.luzonica* - outgroup) specimens were collected

TABLE 1. Specimens used in study

Botanic garden	Collection number	Sequence ref	Origin	floral material collected
Cibodas, Java	LG138	w1	West Java	o
Cibodas, Java	LG141	w3	West Java	o
Cibodas, Java	LG147	w2	West Java	x
Bogor, Java	LG129	e8	East Java	x
Bogor, Java	LG150	w4	West Java	o
Bogor, Java	LG153	w5	West Java	o
Bogor, Java	LG154	w6	West Java	o
Bogor, Java	LG157	c7	Central Java	x
Bogor, Java	LG159	c8	Central Java	x
Bogor, Java	LG163	c9	Central Java	x
Bogor, Java	LG164	w7	West Java	x
Bogor, Java	LG175	e9	East Java	x
Purwodadi, Java	LG177	e1	East Java	o
Purwodadi, Java	LG181	e2	East Java	o
Purwodadi, Java	LG182	e3	East Java	o
Purwodadi, Java	LG183	e4	East Java	o
Purwodadi, Java	LG184	e5	East Java	o
Purwodadi, Java	LG185	e6	East Java	o
Purwodadi, Java	LG186	e7	East Java	o
Purwodadi, Java	LG230	c1	Central Java	o
Purwodadi, Java	LG231	c2	Central Java	o
Purwodadi, Java	LG232	c3	Central Java	o
Purwodadi, Java	LG233	c4	Central Java	o
Purwodadi, Java	LG234	c5	Central Java	o
Purwodadi, Java	LG235	c6	Central Java	o
Eka Karya, Bali	LG242	b1	Bali	o
Eka Karya, Bali	LG243	b2	Bali	o
Eka Karya, Bali	LG244	b3	Bali	o
Eka Karya, Bali	LG245	b4	Bali	x
Eka Karya, Bali	LG246	b5	Bali	o
Eka Karya, Bali	LG247	b6	Bali	o
Eka Karya, Bali	LG249	b7	Bali	x
Eka Karya, Bali	LG251	s1	Sulawesi	x
Eka Karya, Bali	LG255	b8	Bali	x

Vanda by the author and by Kocyan (pers. comm. 2006), it had been found that this sequence contains a 'hypervariable' region, with sequences from different species proving to be highly variable and difficult to align, but was considered to be sufficiently variable for a single species population level study.

TABLE 2. Primers used for amplification and sequencing

Primer	Sequence	Author
c	CGAAATCGGTAGACGCTACG	Taberlet *et al.* 1991
f	ATTTGAACTGGTGACACGAG	Taberlet *et al.* 1991

Total cellular DNA was extracted from silica-gel dried leaf material (Chase and Hills, 1991), according to the method of Doyle and Doyle (1987), without an RNA treatment. The PCR programme consisted of 1 min at 94°C (initial denaturation), 35 cycles of 1 min at 94°C (denaturation), 1 min at 50°C (annealing) and 1 min 10 s at 72°C, and 5 min at 72°C final extension. Amplification was performed using 50 µl reactions, with 1 µl of each primer, 5 µl NH_4, 3 µl $MgCl_2$ (2.5 µl for trnL-F), 1 µl dNTPs, 0.2 µl *Taq* polymerase 1 µl DNA (approximately 20-70ng).

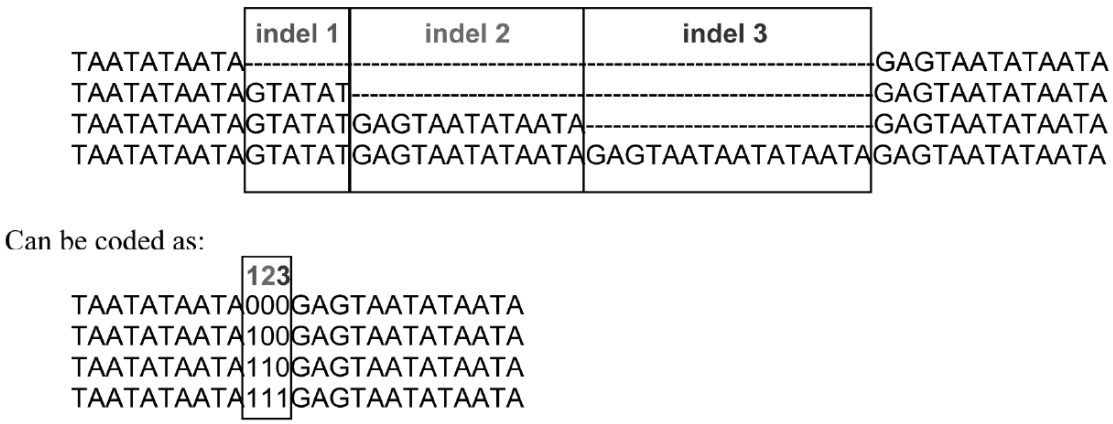

FIGURE 2. Example of coding indels

Amplified products were purified using QIAquick PCR purification kit (Qiagen). Cycle sequencing reactions were performed using Big Dye terminator chemistry (PE Biosystems), under the following conditions: an initial denaturation step at 96°C for 1 min followed by 25 cycles of 10 s at 96°C, 5 s at 50°C, and 4 min at 60°C using 10 µl reactions made with 2 µl Big Dye 3.1, 1 µl Big Dye buffer, 1 µl primer c, and 1 µl DNA (approximately 20-25 ng). Sequences were run on an ABI3700 capillary sequencer (PE Biosystems). Chromas™ and BioEdit™ software programs were used to edit and align sequences, with the initial alignment performed using the Clustal X algorithm in BioEdit™, followed by manual optimisation.

Phylogenetic analysis is not usually used at the population level for a number of reasons. Two reasons are that firstly the lower level of genetic variation within species often means that relationships cannot be resolved due to lack of information in the DNA sequences examined. Secondly, species populations are less likely to be geographically and genetically isolated than distinct species, with gene flow and therefore reticulated relationships possible and even likely. The bifurcating nature and the inherent assumption of non-reticulation and gene flow of phylogenetic analyses means that they are often highly inappropriate for population level analyses. Simple phylogenetic analyses can be carried out however to investigate whether there is strong phylogenetic signal in the data, which might imply reduced gene flow between individuals/populations, and to help guide the investigation.

Neighbour-joining (NJ) and maximum parsimony (MP) analyses were performed using PAUP* version 4.0b10 (Swofford, 2002), with and without rooting with the outgroup, *V.luzonica*. The MP analyses were heuristic searches with 100 replicates, with equal weighting on all characters, ACCTRAN optimization and TBR branch swapping. The initial MP analysis showed that the phylogenetic relationships between the specimens were completely unresolved, and the NJ analysis connected all specimens in a perfect ladder which implies that the relationships are not resolved (the NJ method will always connect taxa step-by-step in this way if there is no phylogenetic resolution, which is supported by the lack of resolution in the MP analysis).

There are several clearly identifiable insertion/deletion events ("indels") in the sequence alignment, these can carry information about phylogenetic relationships which can otherwise be obscured by the individual bases present or absent in those positions. In order to deal with these indels, in a manner which reduces the influence of the individual bases and emphasises the presence/absence of a section of sequence at a particular position, they can be "coded" using various methods. Here the indels were coded simply as presence/absence by the use of an extra character inserted at the end of the alignment for each indel, and the character "0" used to denote the

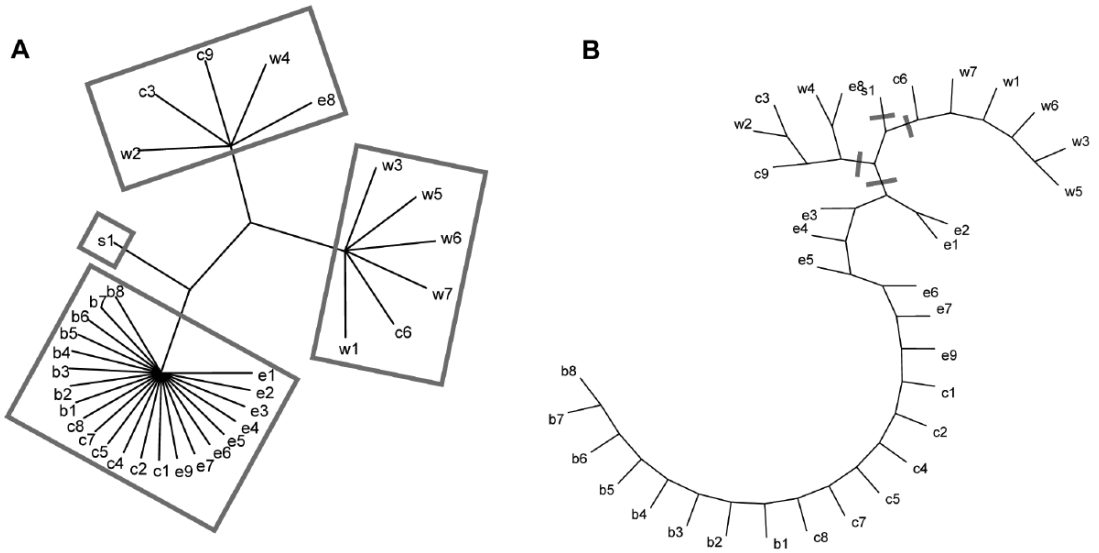

FIGURE 3. Unrooted consensus analyses of *Vanda tricolor* specimens, without outgroup, **A**: MP, **B**: NJ, showing four cor responding clades (denoted by gray boxes/lines)

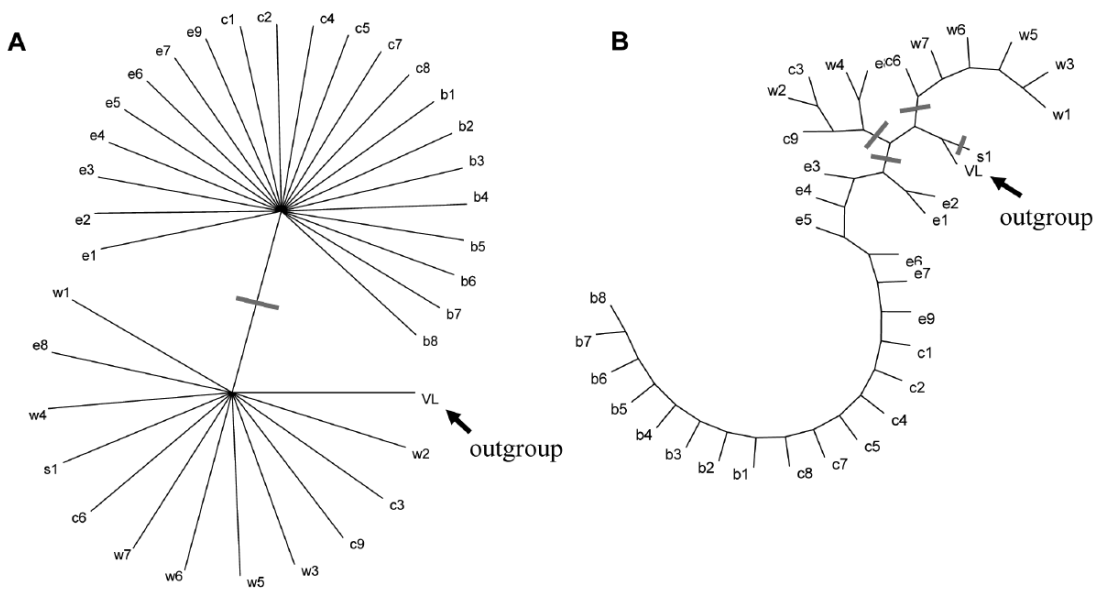

FIGURE 4. Unrooted consensus analyses of Vanda tricolor specimens, including outgroup (VL), A: MP showing only two clades, B: NJ showing four clades previously identified (denoted by gray lines)

absence of sequence at the indel region, and the character "1" to denote the presence of sequence at the indel region, as shown in (Fig. 2).

After coding the indels using this simple presence/absence 0/1 method, the analyses showed much clearer relationships between the specimens. Excluding the outgroup, MP analysis shows the specimens resolved into four clades (as in Fig. 3A) and the NJ analysis shows groupings which correspond to these four clades (Fig. 3B).

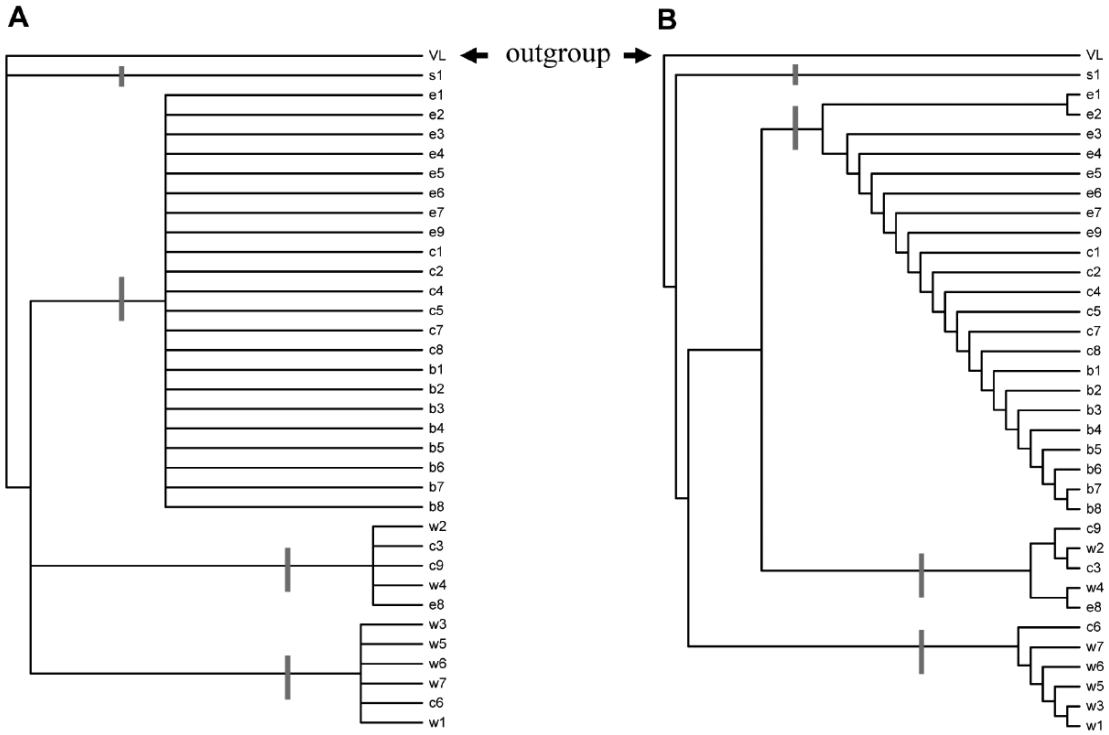

FIGURE 5. Rooted consensus analyses of *Vanda tricolor* specimens (rooted with outgroup, *V.luzonica*), A: MP, B: NJ, showing the four clades also identified in unrooted analyses (indicated by gray boxes/lines)

When the outgroup, *V.luzonica*, was included in the analyses but not used to root them, two clades congruent with the four clades previously seen were present in the MP consensus tree (figure 4A), but all four clades were present in the NJ consensus tree (Fig. 4B). The outgroup appears to be mostly closely related to the *V.tricolor* specimen from Sulawesi, s1, in figure 4B, and with the [w1, w3, w5, w6, w7, c6] clade. Rooting the analyses with the outgroup results in all four clades being resolved in both the MP and the NJ consensus trees, and the outgroup most closely related to specimen s1 (figures 5A and 5B).

The coded sequence alignment was analysed using two programs used for population genetic analyses – TCS (Clement et al 2000) and Network (Bandelt et al 1999). TCS estimates gene genealogies which may include multifurcations and/or reticulations, producing networks using a method of network estimation called Statistical Parsimony (Templeton et al 1992). Statistical parsimony calculates the probability for DNA pairwise differences until the probability exceeds 0.95 ("95% cutoff"), emphasising the shared characters among haplotypes by connecting the most similar haplotypes first, then those with one mutation, then two, and so forth. The number of mutational differences associated with this probability just before the 95% cutoff is the maximum number of mutational connections between pairs of sequences that can be justified by the criterion. TCS only processes sequences using the standard DNA code, so the 0/1 coded characters used previously were converted into ACGT characters prior to TCS analysis. Because TCS uses the differences between the sequences, not the actual bases in the sequence (ie. it does not infer information from *which particular* base is at a position, rather than whether the base is *different* to that in the other sequences at the same position), the 0 and 1 characters were substituted with A, C, G or T. Network uses a method of network estimation called Median Joining, combining minimum spanning trees into a single network using median vectors (nodes) which represent missing intermediate haplotypes, but does rely on the absence of recombination in the data.

Both methods of estimating the genealogy of the

TABLE 3. Specimens used in study

Haplotype	Sequence reference	Origin
1	c1	Central Java
1	c2	Central Java
1	c4	Central Java
1	c5	Central Java
1	c7	Central Java
1	c8	Central Java
1	e1	East Java
1	e2	East Java
1	e3	East Java
1	e4	East Java
1	e5	East Java
1	e6	East Java
1	e7	East Java
1	e9	East Java
1	b1	Bali
1	b2	Bali
1	b3	Bali
1	b4	Bali
1	b5	Bali
1	b6	Bali
1	b7	Bali
1	b8	Bali
2	w2	West Java
2	c3	Central Java
2	c9	Central Java
3	w3	West Java
3	w5	West Java
3	w6	West Java
3	w7	West Java
3	c6	Central Java
4	s1	Sulawesi
5	w4	West Java
6	e8	East Java
7	w1	West Java
8	VL (V.luzonica)	Philippines

specimens produced the same network, collapsing the data into 8 haplotypes (numbered 1-8), the eighth being the *V.luzonica* sequence (Fig. 5). The specimens fall into the haplotypes as listed in Table 3.

The haplotypes are not individually restricted to a single geographic region, but appear to be distributed in a non-random manner (Table 4), with the most haplotypic diversity in West Java (four haplotypes present – 2, 3, 5, 7), the least in Bali and Sulawesi (only one haplotype present in each, 1 and 4 respectively), and haplotype 1 is the most widespread, found in Central and East Java and in Bali.

Both methods of analysis included the same loop which cannot conclusively be broken using the guidelines of Crandall and Templeton (1993) (Fig. 6). Singletons (ie. unique haplotypes or those present at low frequencies) are more likely to be at the tips of networks than internally, so the loop is most likely to be broken at point A or B than at the other two possible places. The unrooted phylogenetic analyses (MP and NJ) showed that haplotypes 8 and 4 (the *V.luzonica* outgroup sequence, VL, and *V.tricolor*, s1) are more closely related to haplotype 3 (w3, w5, w6, w7, c6) than to haplotype 1 (c1, c2, c4, c5, c7, c8, e1, e2, e3, e4, e5, e6, e7, e9, b1, b2, b3, b4, b5, b6, b7, b8), supporting network 5B as the more likely of the two.

The network was subdivided into clades as described in Templeton (1998) (Fig. 7) and Nested Clade Phylogenetic Analysis (NCPA) carried out using the program GeoDis (Posada et al 2000). The results of the contingency analysis of clade 1-3 (equivalent to clade 2-2, and included in clade 3-1 which includes the whole network) is significant at the 5% level, with all other clades being non-significant. When interpreted using Templeton's latest revised inference key (http://darwin.uvigo.es), the outcome of the nested contingency analysis of geographical associations infers that clade 1-3 has been affected by restricted gene flow with isolation by distance. No conclusions can be evaluated from the data for the other clades, probably as a result of low sample size, to which NCPA is sensitive.

The geographic pattern of the *V.tricolor* specimens subdivision and structure was tested by analysis of molecular variance (AMOVA) (Excoffier et al 1992), with ø-statistics calculated analogous to the F-statistics of population genetics. Many different structural hierarchies were assessed, grouping the haplotypes according to geography and phylogenetic clades, and the results showed that the most appropriate subdivision of the haplotypes accounting for 88.66% of the variation among the groups was that which divided the specimens into the four clades found in the phylogenetic and haplotypic analyses, and shown in the network grouped as in figure 8: [s1], [w1, w3, w5, w6, w7, c6], [w4, c3, c9, e8], and [c1, c2, c4, c5, c7, c8, e1, e2, e3, e4, e5, e6, e7, e9, b1, b2, b3, b4, b5, b6, b7, b8].

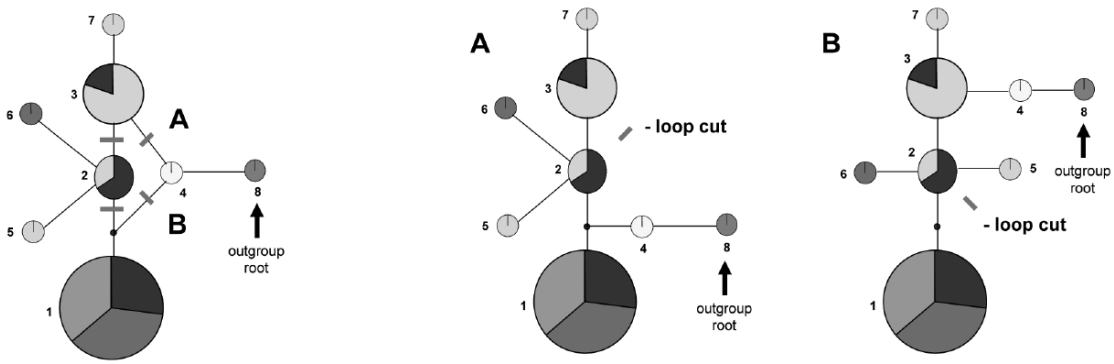

FIGURE 6. Statistical parsimony and Median-joining network, gray bars indicate the four places where the ambiguous loop could be cut, with alternative topologies - A: ambiguous loop cut at position A, B: ambiguous loop cut at position B.

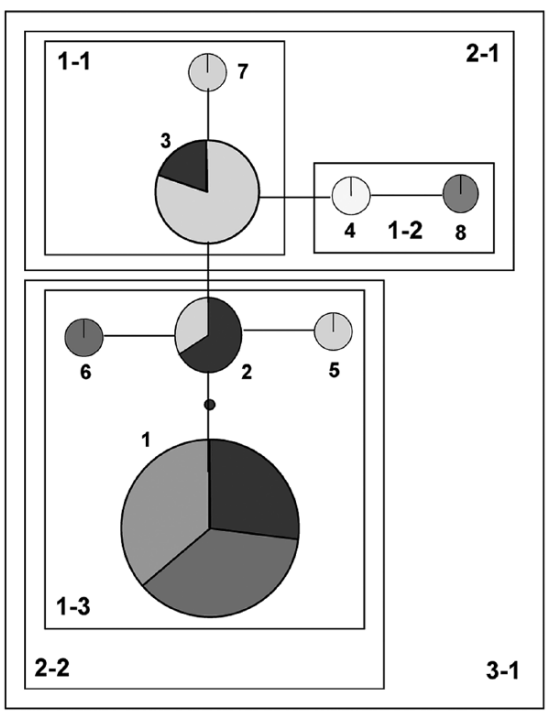

FIGURE 7. Network coded for Nested Clade Phylogenetic Analysis

Discussion

The results suggest that geographically the most recent common ancestor of *V.tricolor* and *V.luzonica* originated in or near to Sulawesi and the Philippines, and colonised West and Central Java first, before spreading into East Java and Bali. As the species spread across the region, mutational change resulted in new haplotypes evolving, so we could predict that haplotype 4 is the oldest *V.tricolor* haplotype, followed by haplotype 3, and then haplotype 2, with the terminal haplotypes 1, 5, 6, 7 being the most recently evolved. These divergences can be used to infer when the species spread from Sulawesi, into West and Central Java, and then into East Java and Bali, using previously published mutational rates of change in chloroplast DNA sequences and information about the sequences used. The method of Saillard et al (2000) was used to calculate these divergence times, and the aligned sequence length of 271 base pairs and the mutation rate of 4.87 x 10^{-10} substitutions per site per year (Richardson et al 2001) were used. The calculated date of the movement of *V.tricolor* from Sulawesi into West and Central Java is 1,894,269

TABLE 4. Specimens used in study

Locality	Number of haplotypes	Total number of individuals	Haplotype (number of individuals)
West Java	4	7	2 (1), 3 (4), 5 (1), 7 (1)
Central Java	3	9	1 (6), 2 (2), 3 (1)
East Java	2	9	1 (8), 6 (1)
Bali 1	8	1 (8)	
Sulawesi	1	1	4 (1)
Philippines (outgroup)	1	1	8 (1)

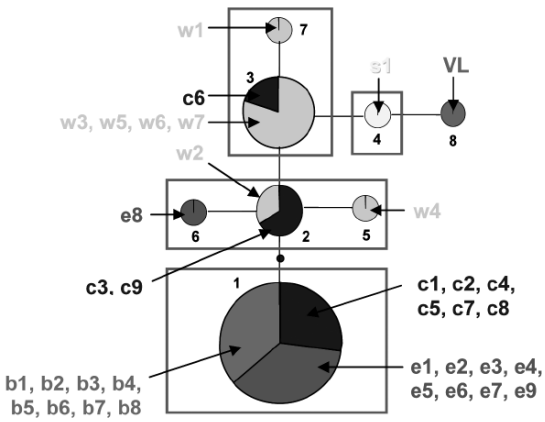

FIGURE 8. Haplotypic network with four clades that account for 88.66% of the variation among groups

years ago +/- 568,281 (between 1,231,275 – 2,462,550 years ago), and from West and Central Java into East Java and Bali is 1,470,844 years ago +/- 134,500 (between 1,336,345 – 1,605,344 years ago). These dates are at the end of and after the Pleistocene, when there was a shallow Java Sea in the region and there is evidence of land bridges which would have facilitated the migration of plants and animals from continental Asia.

The main restriction of this investigation has unfortunately been the relatively low sample size, totalling 34 *V.tricolor* specimens, due to the lack of specimens with collection localities recorded and the lack of populations in the wild available for sampling. As statistical and modelling-based analyses are sensitive to sample sizes, the results must be interpreted with caution. However, the results to suggest an interesting colonisation pattern which may warrant further investigation in this and other species, and shows that the remaining populations of V.tricolor in Java and Bali appear to be at least partially genetically isolated, with widespread haplotypes and others only found in one region.

Conservation efforts to reintroduce and/or increase populations of *V.tricolor* should take into account the diversity of haplotypes in Java and Bali in order to conserve genetic diversity and potential. This study shows that not all specimens of *V.tricolor* in Java and Bali are genetically identical and that different regions have different genetic varieties. The next stage in this investigation will examine the floral

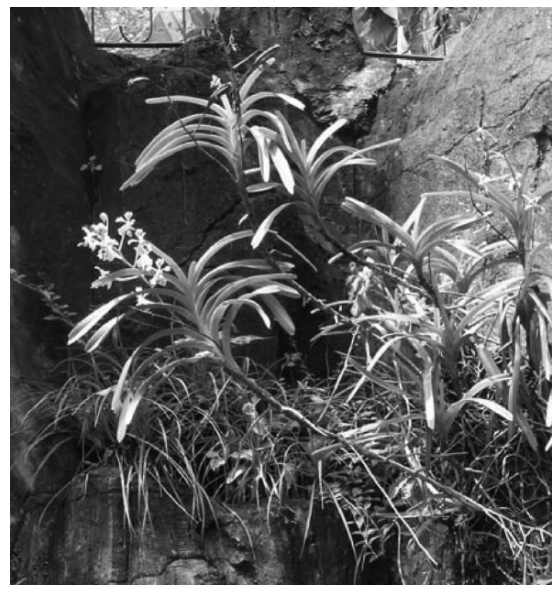

FIGURE 9. *Vanda tricolor* var *suavis* growing in Purwodadi Botanic Garden, Java

morphology of the specimens using the spirit collection material collected at the same time as the DNA material, and determine whether there is any relationship between morphological characters observed in the flowers and their genetic and/or geographic structure. There is taxonomic confusion about the status of species/varieties/subspecies such as varieties *suavis*, *planilaberis* and *purpurea*, which may be clarified when examined in relation to the genetics and geographic origins of the taxa.

The specimens from the reintroduction efforts on Mount Merapi, Java, are genetically diverse, with the three most common haplotypes found making up the sample, conserving more genetic potential than if the specimens all belonged to the same haplotype. It could be advised that efforts should be made to self-pollinate these plants in order to preserve the genetic potential, rather than to cross-pollinate them with each other whilst they are in cultivation and before they are reintroduced into the national park, and even to reintroduce plants into geographically separate sites in the park to reduce natural cross-pollination.

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LITERATURE CITED

Bandelt H. J., P. Forster & A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molec. Biol. Evol. 16: 37-48.

Chase, M.W. & H.G. Hills. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. Taxon 40: 215-215.

Clement, M., D. Posada & K.A Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9 (10): 1657-1660.

Crandall, K.A. & A.R. Templeton. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. Genetics 134: 959-969.

Doyle, J.J. & J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of leaf tissue. Phytochem. Bull. 19: 11-11.

Posada, D., K.A. Crandall & A.R. Templeton. 2000. GeoDis: A program for the cladistic nested analysis of the geographic distribution of genetic haplotypes. Mol. Ecol. 9: 487-488.

Saillard, J., P. Forster, N. Lynnerup, H.J. Bandelt & S. Nørby. 2000. mtDNA variation among Greenland Eskimos: the edge of the Beringian Expansion. Am. J. Human Genetics 67: 718-726.

Swofford, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods) Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Richardson, J.E., R.T. Pennington, T.D. Pennington & P.M. Hollingsworth. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. Science 293: 2242-2245.

Taberlet, P., G. Ludovic, G. Pautou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17: 1105-1109.

Templeton, A.R., K.A. Crandall & C.F. Sing. 1992. A cladistic analysis of phenotypic association with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132: 619-633.

Templeton, A.R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. Mol. Ecol. 7:

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AREA RECOVERY AND CHARACTERISTIC ORCHIDS CONSERVATION "*IN SITU*" AT SAN ANGEL STONY TERRAIN, MEXICO, D. F : RESERVOIR AREA AND ECOLOGICAL PATHWAY AT SOUTH SCIENCES AND HUMANITIES EDUCATIONAL CENTER (SSHEC) WITHIN THE NATIONAL AUTONOMOUS UNIVERSITY OF MEXICO

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RESUMEN. Este trabajo pretende contribuir a la conformación de una Estrategia Global para la Conservación de Orquídeas, a través de una experiencia concreta desarrollada en un Plantel Educativo ubicado al sur de una de las ciudades más grandes y pobladas del planeta, la Ciudad de México, proponiendo como elemento básico el rescate de las áreas verdes que están desaprovechadas y deterioradas y su uso para la conservación "*in situ"* de especies de orquídeas características del lugar. Este Plantel fue construido en la zona denominada " Pedregal de San Ángel", producto del derrame de lava del volcán Xitle, misma que alberga a un ecosistema de matorral xerófito considerado único en el mundo, tanto por su diversidad como por su número de especies endémicas; en el mismo han sido reportadas 22 especies de orquídeas terrestres. Para el año 2000 este ecosistema se había reducido en más del 90%. El Plantel cuenta con 39,039 m^2 de áreas verdes, algunas de las cuales aún conservan especies características de este matorral. En el año 2000 se logró que al interior del mismo se decretara como área de Reserva Ecológica a una superficie de 2710 m^2 que estaba deteriorada; se rescató y se construyó un Sendero Ecológico y se han detectado, estudiado y protegido a 6 especies de orquídeas (*Bletia campanulata*, *Brachystele polyantha, Deiregyne pyramidalis, Dichromanthus aurantiacus, Habenaria novemfida* y *Sarcoglottis schaffneri*), e incluso se han introducido ejemplares de la especie *Malaxis myurus,* provenientes de rescates realizados en zonas aledañas, en los que se ha involucrado a profesores y alumnos del Plantel, obteniéndose excelentes resultados. Asimismo se han diseñado y aplicado Estrategias Didácticas para el conocimiento, valoración y conservación de estas especies de orquídeas.

KEY WORDS: conservation, education, orchids, pathway, recovery, reservoir

Antecedent

ZONE LOCATION.- One of the largest and populated cities on earth, Mexico City, has at present a small amount of trees and shows a green areas vanish annual rate of 3.7% (Ezcurra *et al*. 1990). Southward of this City, immerse in a zone known as "San Angel Stony Terrain", and adjacent to University campus of the National Autonomous University of Mexico (NAUM), it is located South Sciences and Humanities Educational Center (SSHEC) subsidiary baccalaureate stream of the NAUM. Both the Educational Center and the University Campus, were built on the lava thrown out from Xitle volcano, which erupted approximately 2500 years ago rendering a worldwide unique ecosystem due to both its diversity and endemic species amount, besides its peculiar location in Nearctic and Neotropical confluence, and heterogeneous topography caused by lava solidification, thus producing an important microclimates and microhabitats mosaic (Rzedowski 1954, Alvarez *et al*. 1982, Rojo & Rodriguez 2002). In spite of the SSHEC is a typical urban environment, Educational Center become of great interest because it is immerse in a redoubt of this ecosystem and it is possible to find green areas that preserve characteristic species from it (Garduño *et al*. unpubl. 2003).

GENERAL ZONE DESCRIPTION.- "San Angel Stony Terrain" is a large mass of volcanic rock. Until 1950

FIGURE 1. *Senecio praecox*, typical species at San Angel stony terrain.Photograph by Cecilia Garduño.

it was an 80 km^2 area with different vegetal communities, in the lower parts the foremost community was Senecionetum praecosis (Rojo *et al*. 1994). This community is a xerophytic scrub characterized by woody species less than four meters tall, typical in semiarid environments, in which *Senecio praecox* or "palo loco" dominated until a short time ago; the community name is derived from this species (Fig. 1).

Originally, this community occupied 40 km^2, but during the last century it has been reduced to 1.47 km^2, due to factors such as Mexico City's drastic growth, putting a great number of species in extinction danger (Soberon, Rosas & Jimenez, 1991). In spite of the aforesaid, this ecosystem still has an important biodiversity, although it has occur a dynamic change in species composition, it still maintain a high percentage (50 %) of the original components and a high total number of species, as well as endemics (Valiente & Luna, 1994).

At present this community is basically confined to the NAUM Ecological Reservoir area, occupying an extent of 237.1 hectares, and here it has been reported 301 angiosperm species, grouped within 61 families (Valiente & Luna 1994), out of 22 species belong to Orchidaceae family (Tellez 2002). Likewise there are endemics examples such as cactus plant *Mamillaria sanangelensis* and orchid plant *Bletia urbana*, Dressler.

GENERAL DESCRIPTION OF EDUCATIONAL CENTER.- SSHEC is located the utmost southwest within the NAUM Ecological Reservoir area and it is built on a volcanic rock ground with slopes fluctuate between 15 and 40%, which means it is a difficult area to construct buildings and to set diverse kind of installations necessary to its performance. However, at present it has been widely constructed, remaining a small number of native vegetation areas that can be rescued. From a 99,242 m^2 total area (almost 10 hectares) only 39,039 m^2 (40%) correspond to green areas, at present these areas are very disturb. It must be noted that this Educational Center attends a broad community of students, professors, and administrative workers.

Purpose

The present article pretends to contribute to management and conservation of deteriorated native vegetation areas, through knowledge, rescue and "*in situ*" conservation of its species, particularly orchid ones, and development of an Environmental Education towards care and conservation of already mentioned species.

Development

In an effort to rescue and conserve native vegetation from this area and to promote "*in situ*" conservation of typical orchid species, after a diagnosis of the 32 green areas in Educational Center was accomplished (Garduño *et al*. unpubl. 1997), it was given a decree, in year 2000, by which a surface of 2710m^2 was declared as an Ecological Reservoir area, here it was carried out rescue tasks, and an Ecological Pathway was constructed and inaugurated on October, 2003 (Figs. 2 and 3). Parallel to this actions, a second diagnosis of green areas vegetation, in the Educational Center, was made (Garduño *et al*. unpubl. 2003), and present terrestrial orchid species were detected and identified (Garcia *et al*. unpubl. 2003). Likewise, starting from 2003 year to the present time, orchid species in Reservoir area and Ecological

FIGURE 2. Reservoir area before rescue. Photograph by Cecilia Garduño

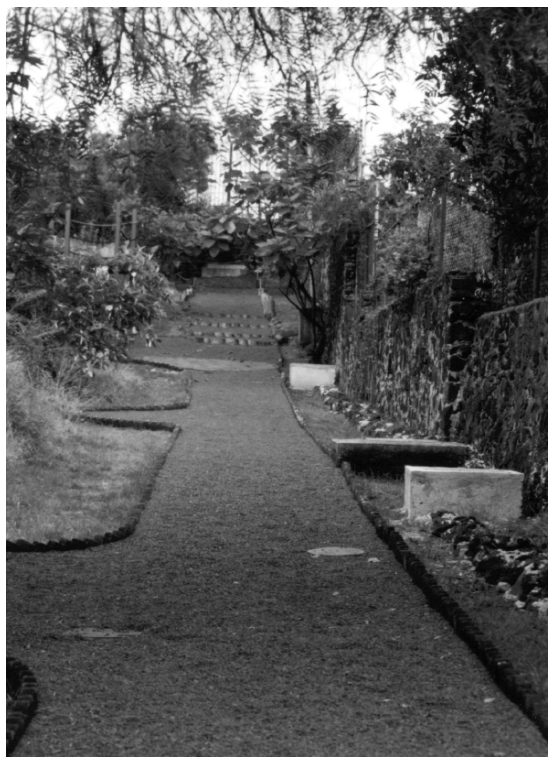

FIGURE 3. Reservoir area and ecological pathway after rescue. Photograph by Cecilia Garduño.

Pathway of Educational Center has been detected, studied and protected, it has participated in rescue and reforestation labors of these species and it has been designed and applied didactic strategy in order to know, to appreciate, and to conserve such unique species.

Results and conclusions

As a result of the investigations related to orchids within the 32 green areas, it was found that in six areas exist seven out of 22 orchid species reported to San Angel stony terrain: *Bletia campanulata* La Llave & Lexarza, *Brachystele polyantha* Reichb. f*, Deiregyne pyramidalis* Lindl*., Dichromanthus aurantiacus* Lex*., Habenaria novemfida* Lindl*., Malaxis myurus (*Lindl.) O.Kuntze*, y Sarcoglottis schaffneri* Reichb. f. (Fig. 4, A-G). Most representative species was *D. pyramidalis* because it was found in five of these areas; followed by *S. schaffneri* y *B. polyantha***,** found in three of these areas and *B. campanulata* found in two. In relation to species care and conservation, actions has been taken involving authorities and gardeners, from the Educational Center, in order to make them sensible about the importance of their participation, resulting in care and "*in situ*" conservation of these species (Fig. 5).

Within Ecological Reservoir and Ecological Pathway, it has been detected, studied and protected, the seven species already mentioned; however, plants of *M. myurus* were not originally found in these areas, those specimens come from rescue activities carried out at University Campus areas bordering Educational Center (Figs. 6, A-B), and were used as a part of reforestation at Ecological Pathway. Professors and students from Educational Center have participated in such activities obtaining excellent results (Fig 7).

Finally, supporting teaching activities of the Educational Center, Didactic Strategies has been designed and applied to professors and students, in order to know, to appreciate, and to conserve these orchid species (Fig.8). In sensible phase, such strategies include video analysis about stony Reservoir vegetation and

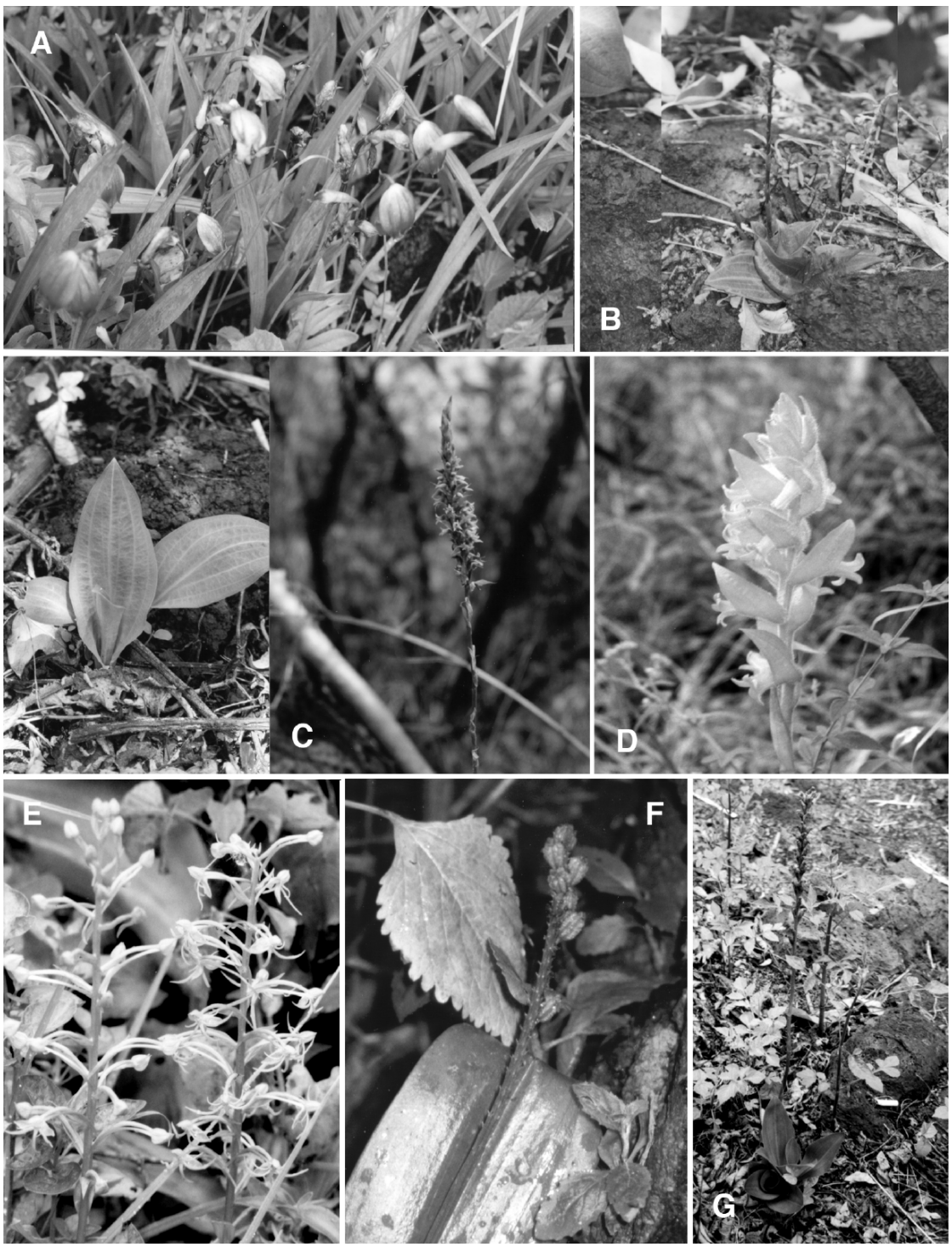

FIGURE 4. A - *Bletia campanulata* La Llave & Lexarza. B - *Brachystele polyantha* Reichb. f. C - *Deiregyne pyramidalis* Lindl. D - *Dichromanthus aurantiacus* Lex. E - *Habenaria novemfida* Lindl. F - *Malaxis myurus (*Lindl.) O.Kuntze. G - *Sarcoglottis schaffneri* Reichb. f. Photographs by Cecilia Garduño.

FIGURE 5. Gardeners working in care and "*in situ*" orchids conservation at Reservoir area.

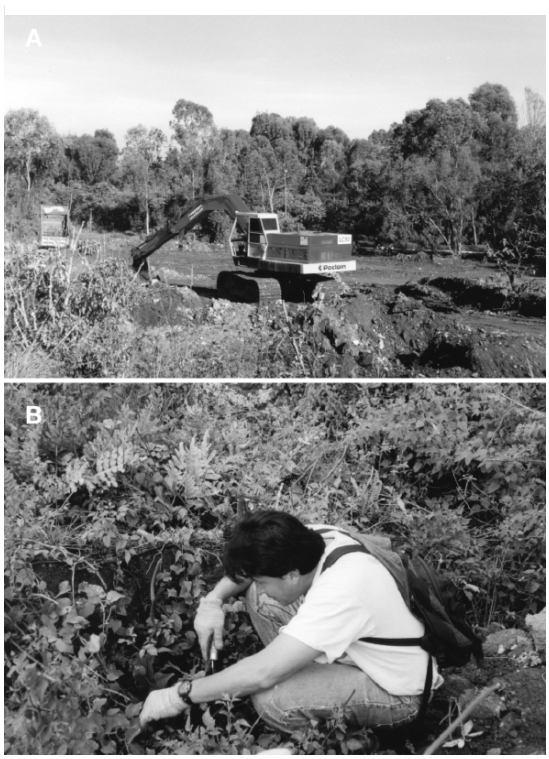

FIGURE 6. A - Green areas reduction at University Campus due to factors such as buildings drastic growth. B - Rescue activities at University Campus areas bordering Educational Center.

conferences about orchids generalities and stony terrain orchids and its importance; in development phase, Reservoir area and Ecological Pathway are examined in order to detect orchids; these activities are complemented with two laboratory experiments about orchids structures and adaptations and, to conclude, written reports about such activities are elaborated as well as group

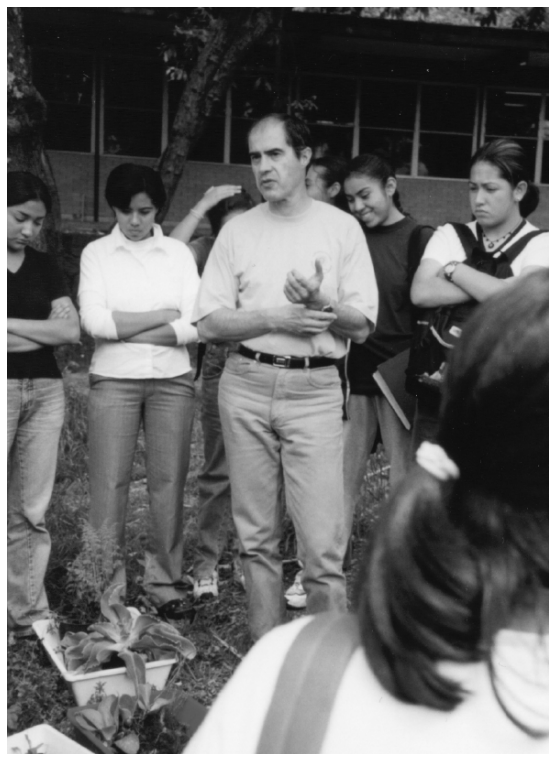

FIGURE 7. Professors and students have participated in reforestation activities at Reservoir area and ecological Pathway.

FIGURE 8. Didactic Strategies has been applied to professors and students, to appreciate and conserve orchid species

analysis and discussion of results.

According to results obtained it can be concluded that deteriorated green areas rescue and its further utilization as Ecological Reservoir areas, are excellent options both to promote knowledge and "*in situ* " conservation of orchid native species, as well as to foment development of an Environmental Education among the community.

LITERATURE CITED

Álvarez, *et al.* 1986. Proyecto para la creación de una reserva en el Pedregal de San Ángel. Cuadernos de Ecología No. 1 Facultad de Ciencias, UNAM. México.

Ezcurra, *et al.*1990. Las ciudades mexicanas del siglo XX. UAM-Xochimilco. México.

García, S. *et al.* unpubl. 2003. Presencia y distribución de especies de orquídeas características del Pedregal de San Ángel, México D. F, en las áreas verdes del plantel sur del C. C. H. Colegio de Ciencias y Humanidades y Jardín Botánico del Instituto de Biología de la UNAM. México.

Garduño, C. *et al.* unpubl. 1997. Diagnóstico de la vegetación de las áreas verdes del Plantel Sur del C. C. H. Seminario SILADIN. Ciencia y Tecnología Ambiental: Ecología Urbana. UNAM. México.

Garduño, C. *et al.* unpubl. 2003. Diagnóstico de la vegetación de las áreas verdes del Plantel Sur del C .C. H. Seminario SILADIN. Ciencia y Tecnología Ambiental: Ecología Urbana. UNAM. México.

Rojo, A. *et al.* 1994. Reserva Ecológica "El Pedregal" de San Ángel: Ecología, historia natural y manejo. UNAM. México.

Rojo, A. & J. Rodríguez. 2002. La flora del Pedregal de San Ángel. INE-SEMARNAP. México.

Rzedowski, J .1954. Vegetación del Pedregal de San Ángel. D .F., México. Esc. Nal. de Ciencias Biológicas, IPN. México. 8 : 59-129 pp.

Rzedowski, J. & G. Rzedowski. 1985. Flora fanerogámica del Valle de México. Vol 2. Publ.15. Instituto de Ecología, A .C. México.

Rzedowski, J. & G. Rzedowski. 2001. Flora fanerogámica del Valle de México. Instituto de Ecología, A .C & Comisión Nacional para el conocimiento y uso de la Biodiversidad. México.

Soberón, J. & J. Carabias. 1994. Prólogo. Reserva Ecológica "El Pedregal" de San Ángel : Ecología, historia natural y manejo. UNAM. México.

Soberón, R. & Jiménez. 1991. Ecología hipotética de la Reserva del Pedregal de San Ángel. Ciencia y Desarrollo. Julio-agosto. México.

Soto, A. 1988. Listado actualizado de las Orquídeas de México. Orquídea (Mex) 11 : 23-27

Soto, A. 1996. Orquídeas mexicanas en peligro de extinción. Boletín de la Sociedad Mexicana de Orquideología A. C. Marzo. México.

Téllez, V. A. 2002. The Pedregal of San Angel and its orchids. The Orchids review 110 (1242) : 25-29.

Valiente, .B & G. Luna. 1994. Una lista florística actualizada para la reserva del pedregal de San Ángel, D. F. Reserva Ecológica del Pedregal de San Ángel. UNAM. México.

Cecilia Garduño was born in Mexico City and was educated at the National Autonomous University of Mexico (UNAM), where she received her Master in Sciences (Biology). She works as biology teacher at South Sciences and Humanities Educational Center within UNAM , 28 years ago, and she has been interested in Environmental Education, particularly in native orchids study and conservation . Among her works are the study and recovery of deteriorated green areas and its usage both as vegetable species Reservoir and Ecological Pathway in order to develop an Environmental Education. She has promoted Orchids Conservation "*in situ*".

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M. A. Aída Téllez was born in Mexico City and received her Master in Sciences (Biology) from National Autonomous University of Mexico (UNAM), where she works as researcher at Botanic Garden within Biology Institute and custodian of the National Collection Orchids, since 1985. She is particularly interested in the Mexican Orchids Conservation. She is also working to several projects about the study and characteristic orchids conservation at "San Angel" stony terrain, Mexico City. She has collaborated and advised to several "*in situ*" conservation orchids projects at Reserve area and Ecological pathway within South Sciences and Humanities Educational Center , UNAM.

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CONSERVATION OF THE GROUP *PIPERIA* (ORCHIDACEAE) AND ASSOCIATED PLANT COMMUNITIES

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KEY WORDS: conservation, habitat, integrated, management, *Piperia*, *Platanthera*

Introduction

The conservation and protection of California native orchids has not been a large focus recently. All California native orchids are terrestrial and many are associated with forest and woodland plant communities. However, a number are associated with the Mediterranean Climate plant community known as Chaparral; this includes at least three *Piperia* Rydb. species. Many *Piperia* populations and associated Chaparral plant communities have been impacted by human activity over the past several decades, however, there is very little documentation regarding the size, and overall impact to the populations.

Piperia contains nine recognized species and two subspecies (Ackerman 1977, Morgan & Ackerman 1990, Morgan & Glicenstein 1993, Hickman 1996) and has been recently placed as a subgenus of *Platanthera* Rich. (Bateman *et al*. 2003). The overall distribution of *Piperia* is fairly large and the group ranges from Baja California, Mexico, to Alaska, and to the northeast, with a single population in Newfoundland, Canada (Fig. 1). Many *Piperia* have limited distributions with populations that are small and disjunct, such as *Piperia elegans* (Lindl.) Rydb. subsp. *decurtata* R. Morgan & Glicenstein (Fig. 2). *Piperia* populations have recently been more fragmented due to human activities such as agriculture, human population expansion, and introduction of noxious weeds.

Preliminary Data

This paper focuses on conservation experiences of rare *Piperia* taxa in California, and highlights the successes and some failures to conserve them. The infor-

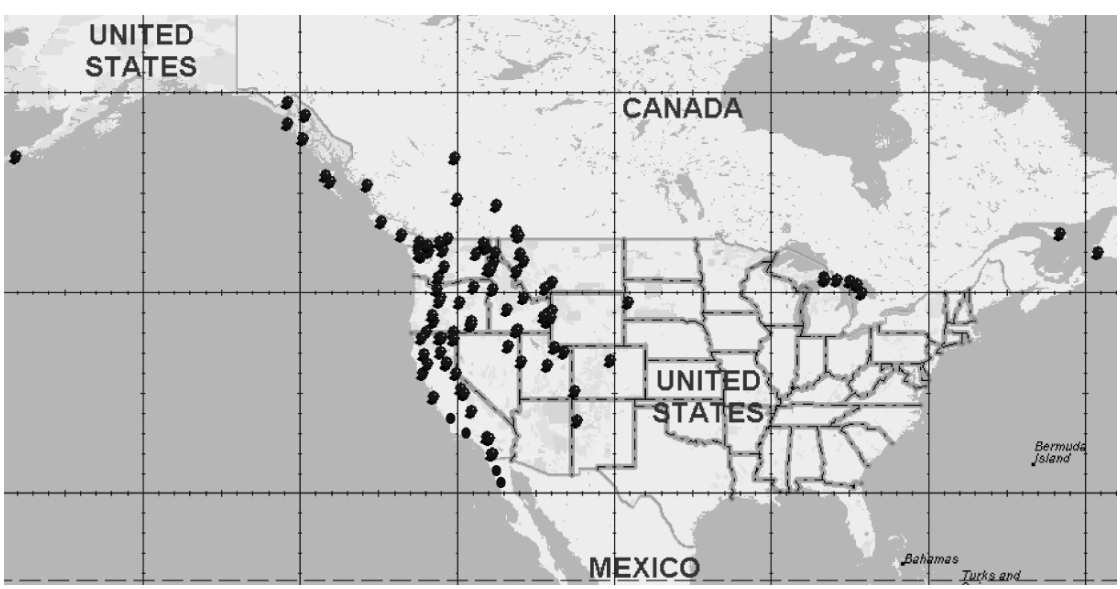

FIGURE 1. Various *Piperia* taxa population distributions.

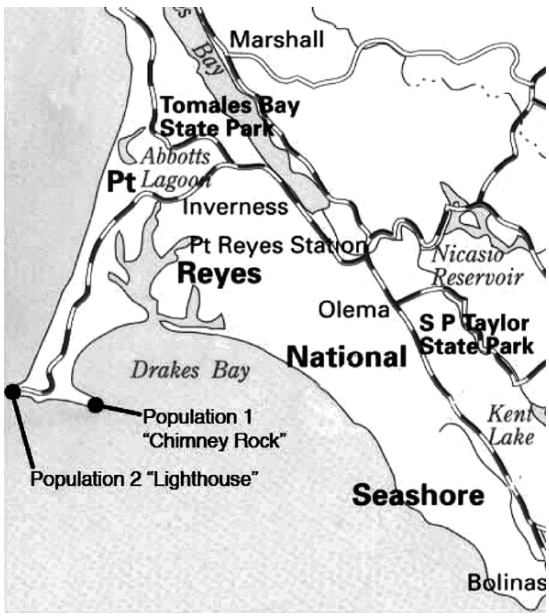

FIGURE 2. *Piperia elegans* subsp. *decurtata* population locations, Marin County, California, USA.

mation presented will be useful for the conservation of most orchids, especially terrestrial taxa. The main focus will be conservation plans for *Piperia cooperi* (S. Watson) Rydb., *P. elegans* ssp. *decurtata*, and *P. yadonii* R. Morgan & Ackerman. The discussion also includes some preliminary molecular data including *ITS* markers used to correctly identify the taxa. Including molecular data can often help to identify taxa and to verify if they are monophyletic. However, not all taxa can be classified by using standard phylogenetic markers and techniques. Providing an integrated approach to conservation of these taxa, including comparative morphology, ecology, and conservation agency cooperation is often also necessary in determining what taxa should be conserved. Using an *ITS* phylogeny for *Piperia* (Fig. 3), we can see that *P. cooperi* is separated from its sister taxa *P. michaelii* (Greene) Rydb. by a moderate bootstrap value of 63, while *Piperia elegans* subsp. *decurtata* doesn't separate out from *P. elegans* (Lindl.) Rydb. subsp. *elegans*, and *P. yadonii* doesn't separate out from *P. elongata* Rydb. This preliminary phylogenetic data suggest that an additional faster evolving marker, or other information should be included to separate out these taxa. This is why the inclusion of morphological, ecological, and population data is also important in determining the conservation status of rare orchid taxa.

To provide a better understanding of the relationship of these orchids will therefore provide additional data to separate out the taxa for conservation purposes. This is important because without additional data, such as population level, morphological, and ecological data, these orchids may not have sufficient protection on private lands and/or through conservation agencies. A recent example of this is a large (*ca.* 2000–4000) population of *Piperia cooperi* on Point Loma in San Diego County, California, USA. This population has been documented to be the largest single population of *P. cooperi* known. This population recently was slated to be destroyed due to a grading project on U.S. federal property. This was possible because *P. cooperi* has no federal or state protection and the latest data from the California Native Plant Society (CNPS) noted that the plant is a CNPS List 4.2 plant (Table 1). I was contacted as an orchid expert to provide information to the United States federal government regarding *P. cooperi*. Because of my dissertation study, I had visited a number of the *P. cooperi* populations in southern California, and Baja California (Fig. 4A, B, C). I compiled a list of all the

TABLE 1. Conservation status for three *Piperia* taxa. California Native Plant Society (CNPS) List 1B.1: rare, threatened, or endangered in California and elsewhere, seriously endangered in California; CNPS 4.2: limited distribution (watch list), fairly endangered in California. State rank: S1: less than five occurrences or less than 1000 individuals or less than 2000 acres; S2: 6–20 occurrences or 1000–3000 individuals or 2000–10,000 acres; S3: 21–80 occurrences or 3000–10,000 individuals or 10,000–50,000 acres.

Taxon	CNPS Listing	State Rank	Global Rank	State Listing	Federal Listing
Piperia cooperi	4.2	S3.2	G4	None	None
Piperia elegans subsp. *decurtata*	1B.1 CA - Endemic	S1.1	G4T1	None	None
Piperia yadonii	1B.1 CA - Endemic	S2.1	G2	None	Federally Endangered

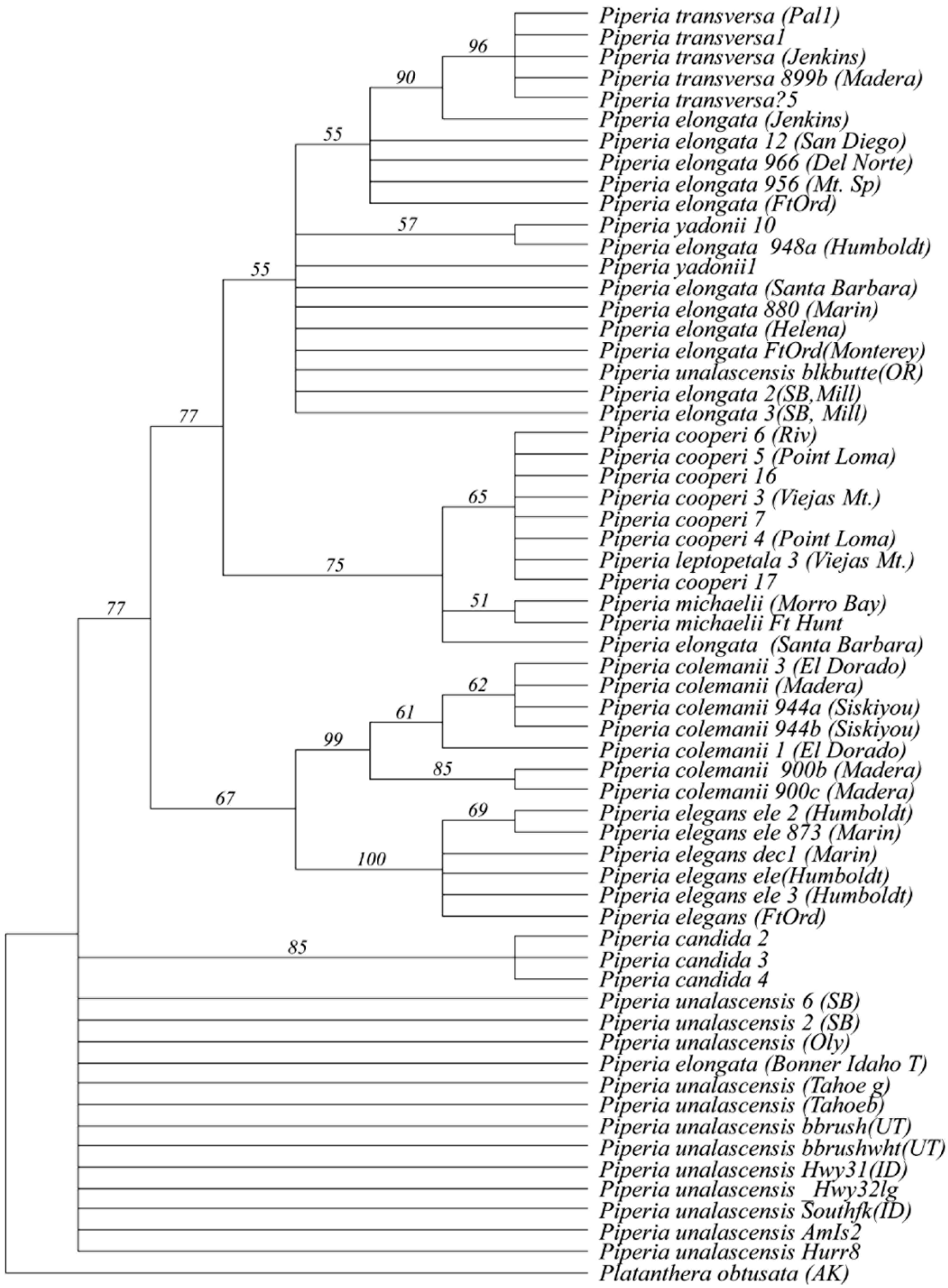

FIGURE 3. *ITS* parsimony bootstrap consensus tree of 63 samples of *Piperia* from California, Baja California, and the western United States

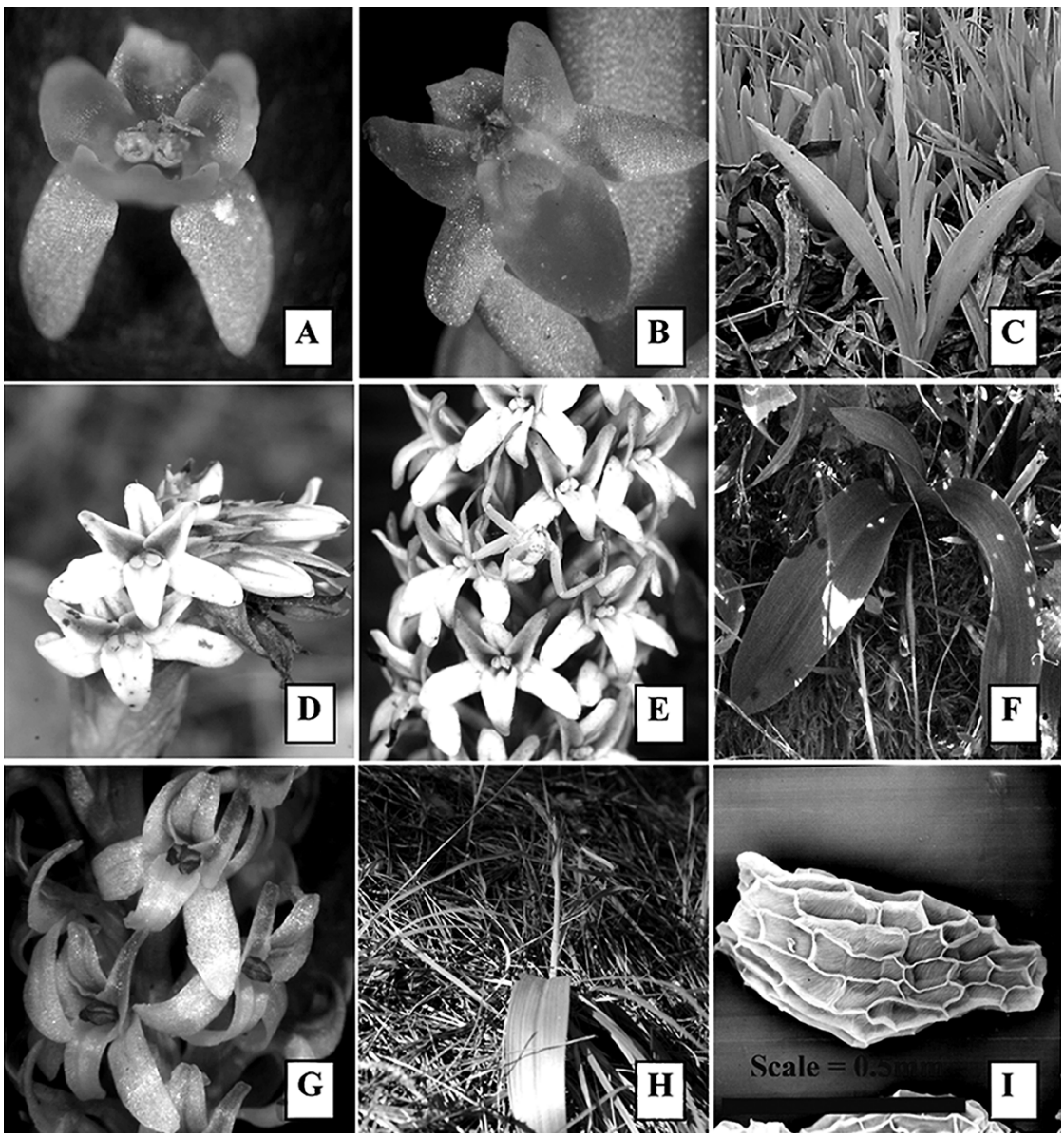

FIGURE 4. *Piperia cooperi* A. Flower (coastal form). B. Flower (inland form). C Leaves. *Piperia elegans* subsp. *decurtata* D. Flower. *Piperia elegans* subsp. *elegans* E. Flower. *Piperia elegans* subsp. *decurtata* F. Leaves. *Piperia yadonii* G. Flower. H. leaves. I. Seed.

known *P. cooperi* populations and cross-referenced these data that I had collected regarding the known populations. I presented the data to the biological representatives of the U.S. federal government noting that of 118 documented populations of *P. cooperi*, approximately 40 could not be found and are presumed not extant due to human impacts. Of the remaining 78 populations, only 50% recently have been re-documented. The vast majority of the populations that have not been seen in over 30 years, though, are presumed extant because the habitat in the area is in good condition. I also provided information that only six newly documented population of *P. cooperi* had been found in the last 30 years. The collection of this data assisted the U.S. federal government in determining that the populations of *P.*

cooperi had been impacted more than was thought from the data previously collected. The botanical community in southern California is now actively searching for historically documented and undocumented populations of *P. cooperi* and counting individuals per population in order to reassess the CNPS and global ranking of this taxon. A request to change the CNPS status of this orchid from CNPS List 4.2 (limited distribution, fairly endangered) to a 3.1 (more information needed; review list, seriously endangered) has been filed with the California Department of Fish and Game.

Piperia elegans subsp. *decurtata* is another rare California orchid that has had some conservation success in recent years. This subspecies of the more common *P. elegans* subsp. *elegans* occurs in two populations within the Point Reyes National Seashore (PRNS) in Marin County, California, USA (Fig. 2). The two subspecies differ morphologically by *P. elegans* subsp. *decurtata* (Fig. 4D) having a short nectary spur (4–6 cm long) when compared with *P. elegans* subsp. *elegans* (Fig. 4E) (7–18 mm long). This taxon was originally given a total number of individuals at approximately 500 (Morgan & Glicenstein 1993). Since that time, a concerted effort to perform annual individual counts has taken place. To this date, the largest number of flowering individuals counted has been 32 in the elephant seal population, and 108 in the lighthouse population (Table 2, R. Lauri, unpubl. data). There are a number of individual *P. elegans* subsp. *decurtata* that have not bloomed in this time period. These individuals have been located during spring surveys when searching for leaf rosettes that are present before bloom (Fig. 4F). During bloom time, the leaf rosettes senesce and are impossible to locate in non-blooming plants. The individual plant counts are conducted to get an accurate count of the total individuals in each population, and to verify the hypothesis that approximately 10–15% of the individuals in a population flower in any given year. The largest single flowering event occurred in 2005, primarily due to an El Niño series of storms that provided the area with twice the annual rainfall. These storms also brought a warm winter season that produced large rosette growth, and an extended rainfall season that allowed greater than normal inflorescence initiation, at the same time there was less impact from by deer as there was more browsing vegetation. The preliminary individual plant counts are not conclusive because the total number of individuals per population or percent bloom of total individuals has not been verified. However, the added interest of the individual plant counts has generated greater involvement within the local Marin Chapter of the California Native Plant Society, who have assisted with the plant counts, and to are continuing to advocate for the conservation of the two populations. The Point Reyes National Seashore also has become more aware of conservation issues surrounding this taxon in conjunction with the need to protect a portion of the elephant seal population during expansion of an access path to the elephant seal overlook. This has caused the initiation of a conservation plan for *P. elegans* subsp. *decurtata* (Lauri, in review), to allow a more active role for all conservation groups in the long-term survival of this taxon.

TABLE 2. *Piperia elegans* subsp. *decrutata* population census data.

Date of Census	Elephant Seal Population	Lighthouse Population
8/31/2000	32	11
8/21/2001	1	1
8/19/2002	3	64
8/19/2003	28	0
8/2004	No data	No data
8/2005	0	108
8/2006	3	4

The only *Piperia* provided Federal Endangered Listing is *Piperia yadonii* (Fig. 4G, H). This *Piperia* is endemic to a very small area of Monterey County, California, USA. Because *P. yadonii* as a restricted endemic range, and is in close proximity to human development, it has been given this special status, even though it is projected that there are over 100,000 individuals (Doak & Graff 2001). Most of the individual orchids are located on private property. *Piperia yadonii* is very closely related to *P. elongata*, and the preliminary *ITS* data does not separate the two taxa (Fig. 3). The *ITS* data also suggests that *P. transversa* Suksd. also is closely related to both *P. yadonii* and *P. elongata*. *P. yadonii* was recently described (Ackerman & Morgan 1990), with an approximate population estimated at 5000 individu-

als. A number of individual plant and population counts have occurred around the Monterey Peninsula. These individual plant counts indicate that from 0.4 to 22% of the vegetative individuals flower in a given year (Graff 2006). This provides us with a total individual orchid count of over 100,000. Even with all populations documented and counted, and the orchids provided Federal Endangered status protection, the local botanical community of Monterey was not satisfied that the orchids were afforded appropriate conservation measures. The United States Fish and Wildlife Service (USFWS) has been compelled to address the conservation of the habitat and plant community that the orchids inhabit. A draft habitat conservation plan has been proposed (USFWS, unpubl.), that addresses the issues of conservation for particular *P. yadonii* populations and surrounding habitat to allow the continued existence and even expansion of this taxon. However, this plan also leaves a number of populations and habitats unprotected due to the U.S. federal government's wish not to over regulate individual landowners and small municipalities. I have reviewed the draft habitat conservation plan and provided the following input to the USFWS. This information is being made public (USFWS, Ventura Office Website 2007). The habitat conservation plan addresses the issue of conserving the largest populations of *P. yadonii* and protecting their habitat, however, there is no information regarding gene flow, specifically the transfer of pollen from one population to the next nor the movement of seed within and between populations. The pollinator of *P. yadonii* are nocturnal moths in the families Pyralidae, Geometridae, Noctuidae, and Pterophoridae (Doak & Graff 2001); however, no study or presentation of data from a Lepidoptera expert has been presented that would address the issue of how far a pollinator can travel and whether there is the possibility of transferring pollen from nearby populations. There also are no data regarding how far *Piperia* seed can travel. It has been hypothesized that the size and shape of orchid seed is conducive to movement by wind and that the vast majority of seed travels only within six meters of the parent plant (Chung *et al*. 2003). This supports the data that most orchid seeds don't remain in the air for long and fall 1.5 m/s in 8 seconds, and suggest that theses seeds are not extremely buoyant (Arditti & Ghani 2000). However, *Piperia* seeds may be fairly buoyant due to the raised cell wall on the seed coat (Fig. 4I). These data suggest that a pattern may occur for *P. yadonii* that is similar to other terrestrial orchid taxa, in that there is low genetic diversity within populations, due to limited seed dispersal, and higher genetic diversity between populations. It is therefore crucial to conserve as many populations of *P. yadonii* as possible in order to retain genetic diversity in all populations of *P. yadonii* and to avoid a population crash due to reduced genetic diversity and gene flow. It has been observed that most *Piperia* produce more viable seed when outcrossed to another individuals, or another taxon, when compared to selfing (Ackerman 1976, Doak & Graff 2001). I have requested a complete study of the population dynamics and population genetics of *P. yadonii* to provide a more complete understanding of the conservation needs of this taxon. This will include individual sampling of specimens from all known populations. In addition, I have requested that an in-depth ecological sampling be taken of the plant communities in and around the *P. yadonii* populations. This will include determining the density and diversity of all vegetation layers associated with the orchid populations, and classification of each of the plant communities found. The ecological habitat study will assist the conservation agencies and landowners in determining a more accurate description of the habitat critical to the conservation of this taxon and associated plant taxa.

Conclusion

These three examples of *Piperia* conservation are good models for continued efforts in terrestrial and epiphytic orchid conservation research. These examples provide instances of initiating conservation goals and collecting evidence to show conservation needs. In addition, once rarity and need have been determined for a taxon, it is clearer what next steps should be taken to conserve the taxa, such as the development of conservation plans followed by annual monitoring of the taxa. Finally, once the taxon has been given protection, there are additional factors to take into account, such as protecting the habitat that the orchid taxon is associated with, as well as specific pollinators. Having molecular data to assist in sepa-

rating out taxa for conservation can be helpful; however, following a total evidence approach to conservation is most successful. This approach can aid in providing additional evidence for the conservation need of orchid taxa, when molecular evidence is inconclusive.

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LITERATURE CITED

Ackerman, J.D. 1976. Biosystematic studies in the genus *Piperia* (Orchidaceae). Master of Arts Thesis, Humboldt State University, Arcata, California, USA.

Ackerman, J.D. 1977. Biosystematics of the genus *Piperia* Rydb. Bot. J. Linn. Soc. 75 : 245–270.

Arditti, J. & A.K.A Ghani 2000. Tansley Review No. 110 Numberical and physical properties of orchid seeds and their biological implications. New Phytol. 145.

Bateman R.M., P.M. Hollingsworth, J. Preston, Y. Luo, A.M. Pridgeon & M.W. Chase. 2003. Molecular phylogenetics and evolution of the Orchidinae and selected Habenariinae (Orchidaceae). Bot. J. Linn. Soc. 142 : 1–40.

Chung, M.Y., J.D. Nason & M.G. Chung. 2004. Spatial genetic structure in populations of the terrestrial orchid *Cephalanthera longibracteata* (Orchidaceae). Amer. J. Bot. 91 : 52–57.

Doak, D.F. & A. Graff. 2001. Reproductive biology and pollination ecology of the federally endangered Yadon's piperia (*Piperia yadonii*, Orchidaceae) in Monterey County, California. Report prepared for the U.S. Fish and Wildlife Service, Ventura Field Office, Ventura, California.

Graff, A. 2006. A long-term monitoring program for the federal endangered Yadon's rein orchid (*Piperia yadonii*, Orchidaceae). Report submitted to the Ventura Fish and Wildlife Office, Ventura, California, USA.

Hickman, J.C. (ed.). 1996. The Jepson Manual: higher plants of California. University of California Press, Berkeley, California, USA.

Morgan, R. & J.D. Ackerman. 1990. Two new *Piperias* (Orchidaceae) from western North America. Lindleyana 5(4) : 205–211.

Morgan, R. & L. Glicenstein. 1993. Additional California taxa in *Piperia* (Orchidaceae). Lindleyana 8(2): 89–95.

United States Fish and Wildlife Service. 2007. Ventura, California, USA. Website available at: http://www.fws.gov/ventura.

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EFFECTS OF TRAMPLING ON A TERRESTRIAL ORCHID ENVIRONMENT

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KEY WORDS: *Cypripedium*, soil compaction, nematodes, nutrient supply

Orchids and soil communities that support them may be affected when we monitor or conduct investigative procedures, or through tourism especially during the blooming season, but this has never been investigated or quantified (Light 2004). The immediate and direct effect of human disturbance including crushing of flowering plants and seedlings is obvious: broken stems are unlikely to resume growth and a season's reproductive effort can be lost. Disturbance of cover vegetation may expose orchids to predators including human poachers. Fragile ecosystems such as bogs and fens may show immediate effects of trampling but forests and grasslands are also vulnerable to foot traffic although these effects may be less apparent especially when traffic is light (Monz *et al*. 2000, Farrior 2005). Malmivaara-Lämäsa and Fritze (2003) reported that the microbial community structure of the humus layer in an urban forest in Finland was affected by a cascade of events beginning with trampling compaction then extending to changes in vegetation and litter quality. A useful quantitative measure of the degree of compaction of mineral soils under forested conditions may be had using a pocket penetrometer which is used to produce a standard indentation (Amacher & O'Neill 2004) while Plant Root Simulator™ - probes (PRS™ probes, Western Ag Innovations Inc., Saskatoon, SK, Canada) have been used to assess all soil nutrient ions simultaneously with minimal disruption to the soil environment (Hangs *et al.* 2002, Hangs *et al.* 2004). Changes in soil nematode populations reflect changes in soil microenvironments and so are increasingly used as monitors of soil health because their numbers, taxa and trophic groups may reflect the state of soil ecological processes (Yeates *et al.* 1993, Neher 2001, Yeates 2003). While nematode numbers may be relatively stable in response to changes in moisture and temperature, their populations are known to respond to land management changes in predictable ways (Yeates 2003). The response to trampling in the vicinity of terrestrial orchids is of interest because it could reflect subtle changes in soil microflora including mycorrhizae which might exert a delayed effect on germination and development of seedlings.

Our long term study of temperate terrestrial orchids has revealed that there can be a lag of two or more years before the effect of natural disturbance or climatic perturbation becomes apparent in terms of plant emergence or flowering (Light *et al.* 2003, Light & MacConaill 2005, 2006). Because many terrestrial orchids have extended periods of below-ground existence as protocorms before emergence, it may require decades of observation to uncover key variables impacting orchid population establishment or stability. This is especially true of long-lived perennials such as cypripediums (Light & MacConaill 2005). We hypothesized that the response of the orchid environment to trampling could include changes in soil compaction, nutrient supply, and the soil nematode community and that these responses could be quantified.

To test these hypotheses, we designed a 30-day experiment where we measured soil temperature, soil moisture, soil nutrient supply, and soil compaction over three 10-day intervals before, during, and after flowering of the shallow-rooted *Cypripedium parviflorum* Salisb. var. *pubescens* Willd. (Knight). The experimental site was in undisturbed forest in Gatineau Park, Québec, Canada (45°30' N, 75°45' W) where the orchids grow in Larose land type Brown Forest soil (Lajoie 1962) developed from glacial till over crystalline limestone (70% sand: pH 6.8–7.1) on a 10% slope facing south (Fig. 1). This

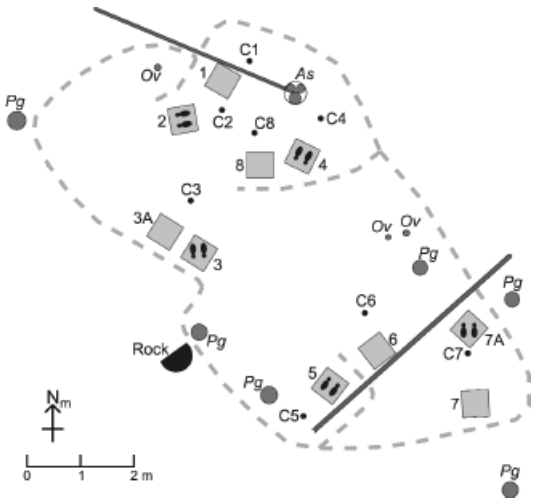

FIGURE 1. Map of study area. Dark grey circles: significant trees surrounding site (*As – Acer saccharum* Marsh.; *Ov – Ostrya virginiana* (Mill.) K. Koch; *Pg – Populus grandidentata*. Black dots: Test plants of *Cypripedium parviflorum* var. *pubescens* (C1–C8). Light grey squares: 50 cm square experimental plots (1–8); foot positions when performing a standard visit indicated. Light grey dashed line: Forest trail.

site is dominated by *Populus grandidentata* Michx. The herbaceous layer consisted primarily of *Erythronium americanum* Ker., *Trillium grandiflorum* (Michx.) Salisb., and *Eurybia (*syn. *Aster) macrophylla* L. Soil depth where the orchids grow rarely exceeded 10 cm. Paired 50 cm x 50 cm plots (5 pairs: 10 plots) were established approximately 50 cm from each of eight multi-stemmed orchid plants. Litter composed of fallen tree leaves covered 80–100% of the plot surfaces. One plot of each pair served as a control and the other was subjected to a daily standard visit during the 10-day blooming period when a 65 kg person wearing soft-soled shoes performed a 5-minute standing visit ("trampling") at a pre-set position facing an orchid plant. The standard visit emulated what a person might do while observing an orchid: feet were not moved during the visit. Shoe type and visitor weight are reported following the approach suggested by Cole (1995). Control plots were monitored but not disturbed during the experiment except to measure soil temperature and to insert/remove the PRS™ probes used to assess soil nutrient supply.

Soil moisture during the 30-day experimental period (May 9 – June 8) averaged 60% of dry matter (range: 35–85; n = 60). Rainfall over this period was 129.4 mm on 21 individual days. Soil remained damp to the touch throughout the experiment. Soil temperature ranged from 9.9 to 18.5°C (mean: 15.3°C over 30 days). Soil compaction (unconfined compressive strength) as measured with a Pocket Penetrometer (Cole-Palmer Model S4651) in the test plots increased steadily over the 10 experimental trampling days (Fig. 2). Footprints became clearly visible beneath covering litter after the first three visits. In the 10-day post-trampling period, there was some recovery from compaction in the test plots, but never to the level of the control plots. We also measured soil compaction along a forest trail that had been created by experimenter traffic adjacent to the plots. We estimate that traffic during the 30 days was equivalent to 50 visitor-passes. This trail showed higher soil compaction than the test plots and this persisted with partial recovery until monthly measurement discontinued at the end of season. Rainfall over the 5-month period (May 9 – Oct 8) was 557 mm on 80 individual days. During the post-experimental period, soil compaction in control and test plots and along the forest trail increased and decreased in parallel suggesting a variable other than trampling alone was affecting the soil environment. While there was no visible change in plant cover of experimental plots, this cover was visibly disrupted along the trail.

Measures of nutrient supply rate in the rhizosphere before, during and after trampling disturbance were made using PRS™ ion exchange probes. Pairs of anion and cation PRS™ probes were inserted in every plot at an angle so that the ion exchange membrane parts of the probes were within the zone where orchid roots might be found, 3 to 5 cm beneath the surface. In the case of experimental plots, PRS™ ion exchange probes were placed just outside the footprint during trampling and within the footprint during the post-trampling phase. Soil nutrient supply rates before trampling ranged from 12–29, 0.8–6.1, and 73–225 $\mu g \cdot (10\ cm^2)^{-1} \cdot (10\ d)^{-1}$ for NH_4^+-N, P, and K, respectively. During the trampling period, soil nutrient supply rates ranged from 5–23, 1.8–7.8, and 76–251 $\mu g \cdot (10\ cm^2)^{-1} \cdot (10\ d)^{-1}$, and during the post-

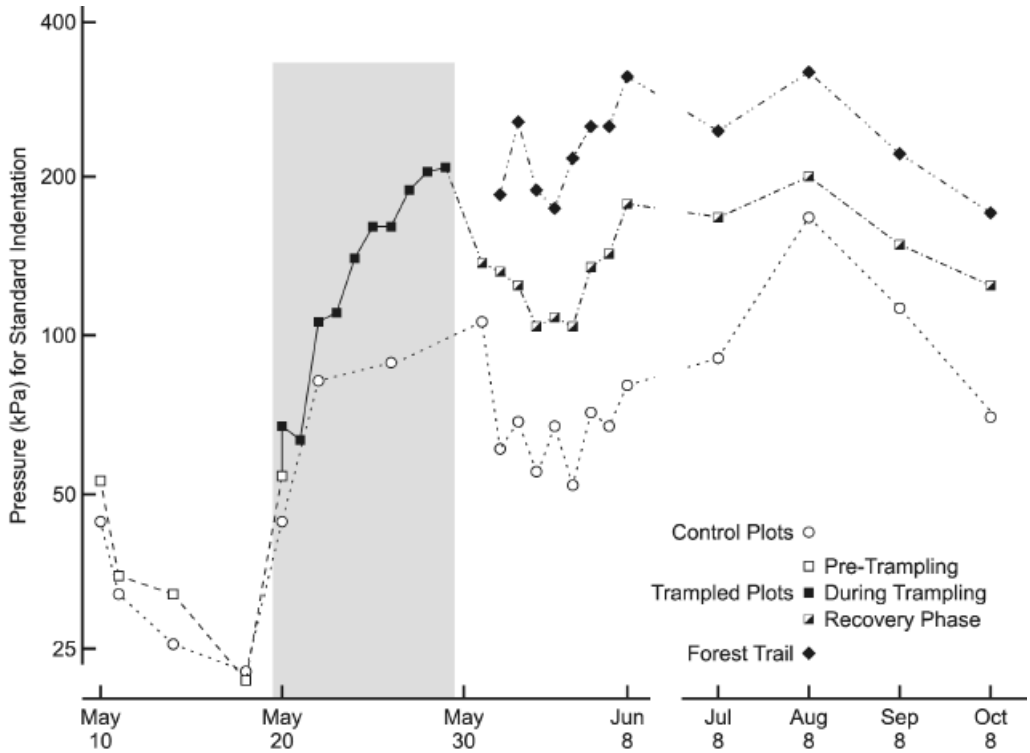

FIGURE 2. Changes in soil compaction, measured as pressure required to produce a standard indentation, before, during and after experimental trampling. The grey band indicates the trampling period: note that compaction was measured immediately before and just after the first trampling visit, and before trampling on the remaining days.

experimental period, rates ranged from 4–24, 1.1–7.9, and 65–131 $\mu g \cdot (10\ cm^2)^{-1} \cdot (10\ d)^{-1}$ for NH_4^+-N, P, and K, respectively. As there was much variation between control data and relatively small overall changes between controls and adjacent test plots, a more profound analysis of soil nutrient supply rates is necessary. This is beyond the scope of the present paper.

Nematode populations are best sampled in the autumn of North temperate regions when populations of free-living nematodes are at their maximum (Boag 1977) . We removed two 5.5 cm dia cores to a depth of 3 cm from each plot and from the adjacent trail on September 8 (3 months after the experiment). In the test plots, we removed the cores from the sole and heel part of one randomly selected footprint. Samples were kept cool until active nematodes were extracted from 100 cm^3 of soil at 22°C room temperature for 48 h using the Baermann Funnel technique (Thorne 1961). Nematodes were killed by gentle heating and examined microscopically for counting and determination of trophic group.

Numbers of nematodes extracted from control and test plots ranged from 17 to 290 per 100 cm^3 soil. Based upon mouthparts and gut structure, we identified representatives of four feeding groups (trophic groups) of nematodes in most samples. Twenty-four different taxa were identified with the greatest diversity found in control plot 6 with 16 taxa. Bacterivorous/omnivorous nematodes were strongly predominant in trampled plots and along the forest trail. Fungivorous nematodes, which feed using a fine stylet to puncture fungal hyphae, were present in most samples but in much lower proportion in the test plots and along the forest trail (Table 1). Enrichment indicator nematode taxa (after Bongers 1990), including representatives of the Rhabditidae and Panagrolaimidae, were present in the trampled areas in greater proportion to basal microbiovores (Table 1). There were few herbivorous nematodes in most plots but somewhat

TABLE 1. Mean±SEM proportions of nematode classes in the various sampling milieus.

	NCR[a]	Enrichment/Total Bacterivores
Trampled Areas[b] (15)	0.91±0.02[c]	0.72±0.06[d]
Control Plots (5)	0.60±0.12	0.16±0.15
Undisturbed Areas (7)	0.49±0.10	0 (all)
All Controls (12)	0.54±0.08[e]	N/A

[a] Nematode Channel Ratio (Moore & Hunt 1988) – Bacterivores/(Bacterivores+Fungivores).
[b] Test Plots + Forest Trail: no differences in proportions between these milieus was discernable.
[c] Significantly greater than All Controls (P<0.001).
[d] Significantly greater than Control Plots (P<0.001).
[e] No significant difference between Undisturbed Areas and Control Plots (P>0.5).

more in forest trail soil samples. Predaceous nematodes (Mononchidae), were present in most samples but in small numbers. To test for any possible effect on the nematode analysis related to the insertion of PRS™ probes, or on the presence/absence of orchids, we sampled soil cores taken from undisturbed sites outside of plot 1 at 10, 25 and 50 cm from Plant 1, and also from sites 5 m to the north, south, east and west of Plants 1, 7, 7, and 3 respectively where no orchids were growing. None of these samples from undisturbed soil contained active enrichment bacterivorous nematodes whereas soils from control plots 3A, 6, and 7 did have some which suggests that the insertion/removal of PRS™ probes may have had a threshold disturbance effect (Table 1).

We have quantified the effect of 10 days (50 minutes total) of trampling on a terrestrial orchid rhizosphere and also of associated experimenter traffic along a forest trail. Soil became compacted and this effect was still discernable four months afterwards (Fig 2). The soil nematode community was noticeably affected by trampling with the appearance of active opportunistic enrichment bacterivorous nematodes (cp value of 1 according to Bongers 1990) and a decrease in fungivorous nematodes, an effect which was equally apparent along the more heavily compacted forest trail (Table 1). Populations of enrichment opportunists can increase rapidly in response to increased nutrient nitrogen availability but may not persist once the enrichment impulse has subsided (Bongers *et al.* 1995). Enrichment indicator nematode taxa are opportunists associated with disturbed conditions (Bongers 1990, Bongers & Ferris 1999, Yeates 2003). They have dormant larval stages which can become active after an appropriate stimulation. Their increase in numbers is considered to be in response to an increase in microbial activity associated with mechanical disruption of litter and increased availability of nutrient substrate (Bongers & Ferris 1999). A preponderance of fungivores and low numbers of active enrichment bacterivorious nematodes would be expected in undisturbed forest (Bongers & Ferris 1999, Neher 2001). "Hot spots" having larger than average numbers of fungivorous nematodes and of total nematodes can also be expected as reflecting the heterogeneity of the forest floor (Moore & de Ruiter 1991). Enrichment opportunists might also appear after natural disturbance events including treefall or animal activity which can create an ephemeral nutrient supply. We could not discern a significant overall effect of trampling in terms of nutrient ion supply but given that there was no consistent decrease in the proportion of fungivorous nematodes in control plots as compared to undisturbed sites (Table 1), we conclude that the use of the PRS™ ion exchange probes was minimally disruptive to this trophic group. It is also possible that any change in nutrient ion supply resulting from our experiment might only become apparent at a later time. The concommitant decrease in fungivorous nematodes in test plots and along the forest trail suggests that their food source, fungal hyphae, has been depleted. This study shows that even small amounts of trampling can have a profound cryptic impact on the terrestrial orchid environment but what this means to the continuity of an orchid population remains to be elucidated.

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LITERATURE CITED

Amacher, M.C. & K.P. O'Neill. 2004. Assessing soil compaction on Forest Inventory & Analysis phase 3 field plots using a pocket penetrometer. Research Paper RMRS-RP-46WWW. Fort Collins, CO: U.S.

Department of Agriculture, Forest Service, Rocky Mountain Research Station. 7 p.

Boag, B. 1977. Factors influencing the occurrence and abundance of nematodes in forest soils in eastern Scotland. Ann. Appl. Biol. 86 : 446–450.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83 : 14–19.

Bongers, T. & H. Ferris. 1999. Nematode community structure as a bioindicator in environmental monitoring. TREE 14 : 224 –228.

Cole, D.N. 1995. Recreational trampling experiments: effects of trampler weight and shoe type. Research Note INT-RN-425. Ogden UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station. 4 p.

Farrior, C. 2005. The effects of social trails on soil compaction and vegetative cover in forest and prairie ecotypes. PennScience 3 : 30–34.

Hangs, R.D., K.J. Greer, C.A. Sulewski & D. Hicks. 2002. Plant Root Simulator™-Probes: An effective alternative for routine soil testing. In Soils and Crops Workshop Proc., pp 120-130. Univ. Saskatchewan.

Hangs, R.D., K.J. Greer & C.A. Sulewski. 2004. The effect of interspecific competition on conifer seedling growth and nitrogen availability measured using ion-exchange membranes. Can. J. For. Res. 34 : 754-761.

Lajoie, P.G. 1962. Soil survey of Gatineau and Pontiac Counties, Québec. Queen's Printer, Ottawa, Canada. 94 p.

Light, M.H.S. 2004. Step softly: environmental stewardship. Native Orchid Conference J. 2 : 29–31.

Light, M.H.S., H. Koopowitz & T.A. Marchant. 2003. The impact of climatic, edaphic and physiographic factors on the population behaviour of selected temperate and tropical orchids. In K. W. Dixon, S. P. Kell, R. I Barrett and P. J. Cribb (eds). Orchid conservation, pp. 159–182. Natural History Publications, Kota Kinabalu, Sabah.

Light, M.H.S. & M. MacConaill. 2005. Long term studies - a case for orchid species survival. Selbyana 26 : 174–188.

Light, M. H. S. & M. MacConaill. 2006. Appearance and disappearance of a weedy orchid, *Epipactis helleborine*. Folia Geobotanica 41 : 77–93.

Malmivaara-Lämäsa, M. & H. Fritze. 2003. Effects of wear and above ground forest site type characteristics on the soil microbial community structure in an urban setting. Plant and Soil 256 : 187-203.

Monz, C.A., T. Pokorny, J. Freilich, S. Kehoe & D. Ayers-Baumeister. 2000. The consequences of trampling disturbance in two vegetation types at the Wyoming Nature Conservancy's Sweetwater River project area. USDA Forest Service Proc. RMRS-P-15. 15 : 153–159.

Moore, J.C. & P.C. de Ruiter. 1991. Temporal and spatial heterogeneity of trophic interactions within below-ground foodwebs. Agriculture, Ecosystems and Environment. 34 : 371–397.

Moore, J.C. & H.W. Hunt. 1988. Resource compartmentation and the stability of real ecosystems. Nature 333 : 261–263.

Neher, D.A. 2001. Role of nematodes in soil health and their use as indicators. J. Nematol. 33 : 161–168.

Thorne, G. 1961. Principles of nematology. McGraw-Hill, New York.

Yeates, G.W., T. Bongers, R.G.M. de Goede, D.W. Freckman & S.S. Georgieva. 1993. Feeding habits in soil nematode families and genera – an outline for soil ecologists. J. Nematol. 25 : 315–331.

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ORCHIDS' MICROPROPAGATION FOR TO THE SUSTAINABLE MANAGEMENT OF NATIVE SPECIES FROM PARQUE NACIONAL Y ÁREA NATURAL DE MANEJO INTEGRADO COTAPATA (PN-ANMI COTAPATA), LA PAZ-BOLIVIA

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KEY WORDS: orchids, *in vitro* germination, micropropagation, Yungas Mountain Forest, culture media, conservation

Introduction

Bolivia is one of eleven countries with the highest biodiversity in earth, due to its variety of ecological belts, ecotones, biogeographic affinities, heterogenic habitats and total species number (Ibish 1996). Concerning to flora, approximately 20,000 angiosperms species have been registered (Beck 1998) and 1,500 of them are included in the *Orchidaceae* family. The region with the highest orchid diversity corresponds to the Yungas Mountain Forest which covers 4% of the national extension and has 60% of the species, being 80% of them endemic of the zone (Vásquez, 2004). In Bolivia, this group is considered as a priority for conservation since many species have some degree of threat, mainly, due to habitat destruction and selective extraction (Vásquez, 2000). The integration of activities focuses in conservation of these resources within native areas and the development of countryside populations is one of the main challenges to prevent the loss of biodiversity. In this sense *in vitro* culture techniques provide an alternative for sustainable management of this kind of natural resources. Through these, it's possible to obtain high amounts of plants for trade purposes, diminishing the pressure on the wild populations.

The project "Estudio del potencial de aprovechamiento sostenible de epifitas en el Parque Nacional y Área Natural de Manejo Integrado Cotapata (PN-ANMI Cotapata)" supported by Fondo Flamenco para el Bosque Tropical, has been developing diverse methodologies for the micropropagation of native orchids from the Bolivian Yungas. The plants obtained *in vitro* will be taken to a communal greenhouse located in "El Chairo" town for their acclimatization. The obtained income will be used to improve economy of the population located in PN-ANMI Cotapata. The presented data constitutes the preliminary results of a 3 years research.

Objectives

- Micropropagation of native orchid species from PN-ANMI Cotapata
- Evaluation of *in vitro* germinative response of 10 orchid species
- Comparison of the species' response under two germination treatments (media culture)

Methodology

Seeds were disinfected in 0.5% sodium hypochlorite (commercial solution) with two drops of a tensoactive agent during 18 minutes. The media culture used were MS (Murashige & Skoog 1962) and KC (Knudson C 1946) supplemented with 15% coconut milk, distributed in culture tubes (160 x 10mm.). This treatment was standarizated after preliminary test based on the protocol described by Villegas (2003). The evaluation of the *in vitro* germination was made six weeks after the introduction, quantifying the proportion of seeds that have reached any of the different germination stages based on the Pierik's germination criteria (1987) modified by Villegas (2003). Once the germinative process evaluation was finished, the

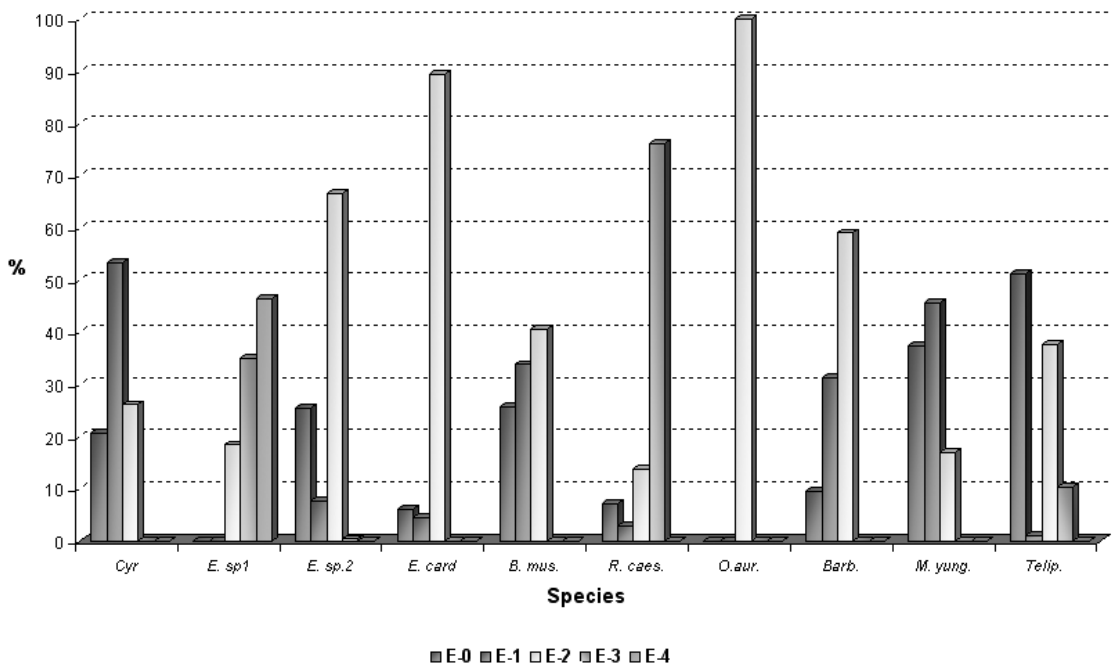

FIGURE 1. *In vitro* germinative process of 10 species of orchids in MS+15% L.C medium, after six weeks of evaluation. *Cyr. Cyrtochilum* sp., *E.* sp1. *Epidendrum* sp. 1, *E.* sp2. *Epidendrum* sp.2, *E. card. Epidendrum cardenasii, B. mus. Brachyonidium muscosum, R. case. Rusbyella caespitosa, O.aur. Odontoglossum aureum, Barb. Barbosella* sp., *M. yung. Masdevallia yungasensis, Telip. Telipogon* sp.

in vitro germinated species were transfered to MS medium without growth regulators for its further growth and development.

The studied species were: *Bracchionidium muscosum* Luer & Vásquez, *Epidendrum cardenasii* Hágsater, *Masdevallia yungasensis* Hashimoto, *Odontoglossum aureum* (Lindl.) Garay, *Rusbyella caespitosa* Rolfe, *Barbosella* sp. Schltr., *Cyrtochilum* sp. Khunt. *Epidendrum* L, *Epidendrum* L and *Telipogon* sp. Khunt. It is important to mention that for the last five cases, at this moment there is no taxonomic determination at species level, since two of them (*Cyrtochilum* sp. and *Telipogon* sp.) could be new species (R. Vásquez, pers. comm. 2006) while in the other cases the bloom time has been expected for their determination.

Results

After a six weeks evaluation, the most advanced stage of germination in both media culture was E-4 (protocorm with foliar primordial) seen only in *Epidendrum* (*E.* sp.1); in this species, the highest rate of germination was registered in KC medium (76.1%) in comparison with MS medium (46.5%). For the stage E-3 (protocorm), a favorable response was registered in KC medium too, since five of ten species studied reached it, while in MS medium only three species reached stage E-3. On the other hand, the species *Cyrtochilum* sp., *Bracchionidium muscosum, Odontoglossum aureum* and *Barbosella* sp. only reached stage E-2. In general, the highest rate of species in stage E-2 was observed in KC medium; however some species showed a more favorable response in MS medium (i.e. *Odontoglossum aureum* and *Barbosella* sp.). For stage E-0 (seeds that have not started the germinative process) *Masdevallia yungasensis* and *Telipogon* sp. showed highest rate in both media, showing a higher proportion in MS medium (Figures 1 and 2).

The results suggest that KC medium with 15% coconut milk is appropriate for the *in vitro* germination of the species studied in general, since in this medium the most advanced developmental stages (E-3 and E-4) were registered (Figure 3). Due to its chemical composition KC medium has been widely used for the *in vitro* orchids' germination (Pierik,

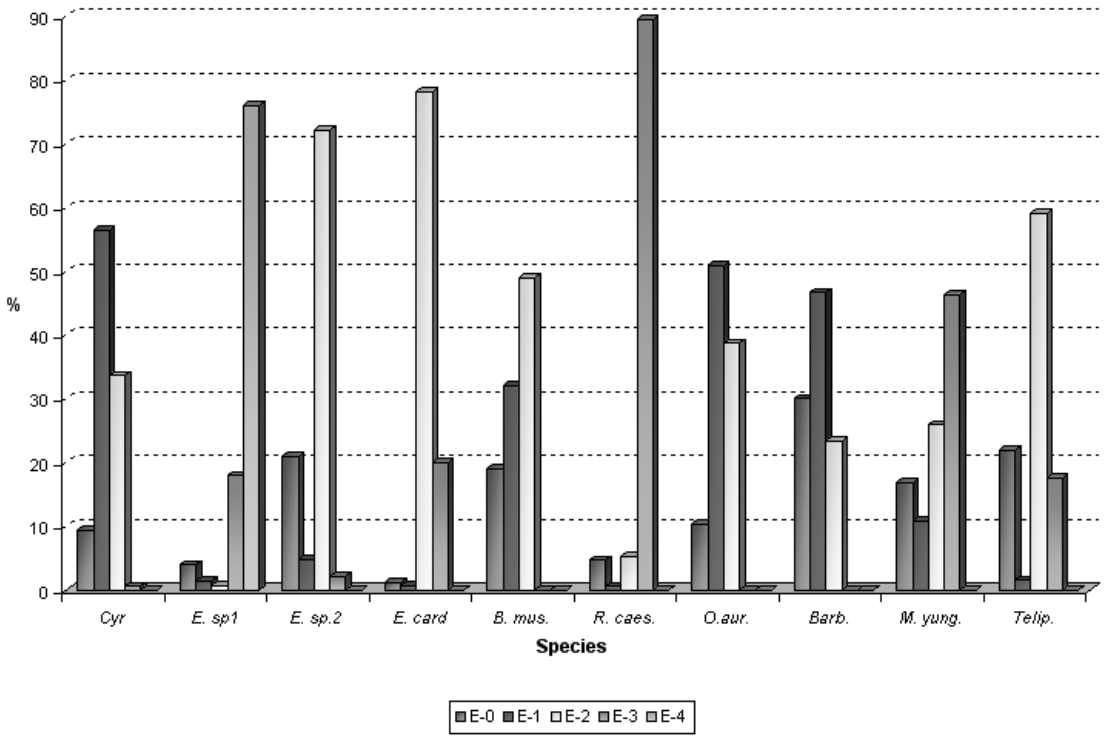

FIGURE 2. *In vitro* germinative process of 10 species of orchids in KC+15% L.C. medium, after six weeks of evaluation. *Cyr. Cyrtochilum* sp*., E.* sp1. *Epidendrum* sp.1*, E.* sp2. *Epidendrum* sp.*2, E. card. Epidendrum cardenasii, B. mus. Brachyonidium muscosum, R. case. Rusbyella caespitosa, O.aur. Odontoglossum aureum, Barb. Barbosella* sp*., M. yung. Masdevallia yungasensis, Telip. Telipogon* sp.

1987; Sánchez, 2006), however the present work showed that some species (*Odontoglossum aureum* and *Barbosella* sp.) have a more favorable response in MS medium according to the results obtained by Villegas (2003) in *Masdevallia chaparensis.*

At this time, the project "Estudio del potencial de aprovechamiento sostenible de epifitas en el PN-ANMI Cotapata" has at least 15 species in germination (without including those reported in this work), 30 in elongation and differentiation, 15 in multiplication and 3 in rooting process. The last ones are ready for acclimatization.

Conclusions

Usually, the most advanced stages (E-3 and E-4) were registered in KC medium.

Epidendrum sp.1 was the species with the best *in vitro* germinative response, since its seeds reached the stage E-4 (protocorms with foliar primordia).

The species *Odontoglossum aureum and Barbosella* sp. showed a more favorable germinative response in MS medium, in contrast to the other species studied.

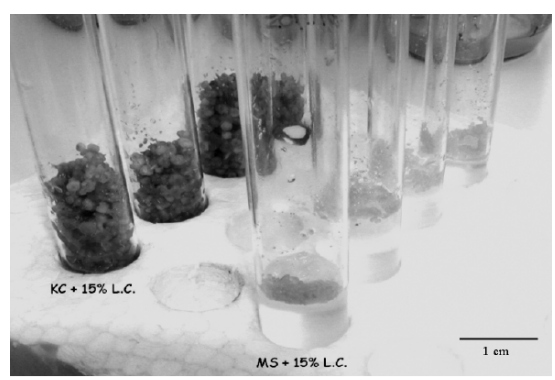

FIGURE 3. Effect of the medium composition on the germination of different orchids' species cultivated *in vitro*. MS+15% L.C: Murashige & Skoog (1962) medium supplemented with 15% of coconut water. KC+15% L.C.: Knudson C (1947) medium supplemented with 15% of coconut water.

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LITERATURE CITED

Beck, S.G.1998. Floristic inventory of Bolivia - An indispensable contribution to sustainable development In: Barthlott, W. & M. Winiger (eds.). Biodiversity – A challenge for development research and policy. Springer – Verlag, Berlin: 243-268.

Ibisch, P. 1996 Neotropische Epiphytendivesität-das Beispiel Bolivien. Wiehl 1996.

Pierik, 1987. *In vitro* cultura of Higher Plants. Martinus Nijhoff Publishers. Dordrecht (Netherland). 344 pp.

Sánchez, A. 2006. Evaluación de diferentes tratamientos para la germinación *in vitro* de semillas de *Masdevallia exquisita* (Pleurothallinidae) del bosque montano de Yungas-Bolivia. Tesis de Grado Lic. Biología. Universidad Mayor de San Andrés. La Paz, Bolivia. 105 pp.

Vásquez, H. R. & P.L. Ibisch, Ed. 2004. Orquídeas de Bolivia, diversidad y estado de conservación. Vol II. Subtribu Laeliinae, Polystachiinae y Sobraliinae, con actualización y complementación de Pleurothallidinae" 2da edición español. Ed. FAN. Santa Cruz-Bolivia. 649 pp.

Vásquez, H., R. & P.L. Ibisch, 2000. Orquídeas de Bolivia, diversidad y estado de conservación . Vol I. Subtribu Pleurothallidinae. Ed. FAN. Santa Cruz-Bolivia. 649 pp.

Vásquez, R. 2006. Curso de Biología, ecología y conservación de Orquídeas (entrevista) La Paz, Bolivia.

Villegas, G. 2003. Evaluación del comportamiento *in vitro* de semillas de *Masdevalia chaparensis* en respuesta a diferentes tratamientos germinativos. Tesis de Grado Lic. Biología. Universidad Mayor de San Andrés. La Paz, Bolivia 99 pp.

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AN EXPANDED ROLE FOR *IN VITRO* SYMBIOTIC SEED GERMINATION AS A CONSERVATION TOOL: TWO CASE STUDIES IN NORTH AMERICA (*PLATANTHERA LEUCOPHAEA* AND *EPIDENDRUM NOCTURNUM*)

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Interest in using mycorrhizal fungi to cultivate orchids from seed *in vitro* (=symbiotic seed germination) has intensified in recent years and this approach is now an important conservation tool worldwide. In North America, symbiotic germination has been attempted for a growing number of orchid species in peril as a means to acquire seedlings suitable for reintroduction. Several taxa have proven surprisingly easy to cultivate in this manner to a leaf-bearing stage, including terrestrials and epiphytes alike (Table 1), and a few have been reintroduced with success. Some, however, have been more problematic. For example, the prairie-fringed orchids of the Midwest (*Platanthera leucophaea* (Nuttall) Lindl. and *P. praeclara* Sheviak & Bowles) require cold-moist stratification to prompt seed germination and development resulting in leaf-bearing seedlings (Zettler *et al.* 2001, 2005; Sharma *et al.* 2003), but seedling survival *ex vitro* has not been achieved. A few species have resisted symbiotic germination altogether despite vigorous attempts (*e.g.*, *Isotria medeoloides* (Pursh.) Raf., Zettler unpubl. data) and may be candidates for alternative techniques (*e.g.*, asymbiotic germination) exemplified by the hardy lady's slipper orchids (genus *Cypripedium*) which have been cultured without fungi.

Although modest progress continues, most of the remaining 200+ species native to the United States and Canada still remain vulnerable because so few have been propagated from seed, with or without fungi. In light of ongoing habitat loss and other ecological concerns (*e.g.*, global warming), it seems likely that a significant number of species will face imminent extinction this century unless conservation efforts are swiftly expanded and rendered more effective. Thus far, symbiotic germination does show promise as an effective conservation tool in North America, especially if it is applied within the framework of integrated and responsible conservation practices that continue to be developed here (*e.g.*, Stewart 2003) and overseas (*e.g.*, Batty *et al.* 2006a, 2006b). Recently, questions concerning the ethics of releasing fungus-infected seedlings "ad hoc" have been raised. For example, the release of laboratory-grown seedlings into the natural habitat has the potential to alter the gene pool of the resident orchid, assuming that such seedlings eventually initiate anthesis (Zettler 2005). Moreover, if the fungus that was utilized *in vitro* originated from a different geographical area or habitat, the act of releasing such a fungus could alter the delicate ecosystem already in place (Zettler 2005). If true, reintroducing orchid seedlings in the "spirit" of conservation could, in fact, prove detrimental. Consequently, the recovery and use of fungi from the original habitat seems preferable to using fungi from distant geographical areas and from different host orchids, except in unusual instances (see Zettler *et al.* 2006 for *Platanthera holochila* in Hawaii).

Gaining an understanding of the fungal associates *in situ* and their role on the orchid life cycle is crucial, and is an important conservation objective for a number of rare North American orchids (*e.g.*, *Platanthera leucophaea*; Bowles & Bell 1999). In nature, orchid protocorms, seedlings and mature plants are assumed to associate mostly with higher fungi, especially members of the Phylum Basidiomycota (= club fungi or basidiomycetes), based on standard fungal isolation techniques (*e.g.*, Currah *et al.* 1997) and direct PCR amplification of fungal genes from orchid tissues (*e.g.*, Shefferson *et al.* 2005). Orchids also associate with members of the Phylum Ascomycota (= sac fungi or ascomycetes), conidial fungi (= "Fungi Imperfecti" or

molds), and vesicular-arbuscular mycorrhiza (Currah *et al*. 1997), but the extent of their physiological role(s) is uncertain. Currah *et al.* (1997) suggested that some of the ascomycete and mold associates – largely discounted by researchers – may actually be equally important to orchids *in situ* because of their nutrient-gathering and sequestering roles. Thus, a wider range of fungal groups may be at play within the greater rhizosphere than simply those that form distinctive intracellular pelotons (*i.e*., the basidiomycetes). Until reliable protocols can be developed to ascertain the role(s) of these seemingly less important fungal groups, current emphasis will remain on the peloton-forming fungi. During the past decade, the role of *in vitro* symbiotic germination has expanded beyond simple propagation. Many researchers now utilize *in vitro* symbiotic germination as a tool to augment molecular studies by verifying the mycorrhizal nature of the fungi identified. As more orchid taxa are studied in this manner and over a wider geographical area, the natural distribution and identity of the physiologically significant fungal strains becomes more apparent. Such data are invaluable at answering questions aimed at fungal specificity and orchid distribution – both of which play a vital role in fostering effective orchid conservation. *In vitro* symbiotic germination also continues to have a useful role in answering questions aimed at fundamental orchid biology. The "closed system" or microcosm offered by a Petri plate, agar medium, and fungus allows for careful manipulation of the factors that influence seeds and seedlings (*e.g*., light pretreatment, substrate pH, seed stratification). Such experimental outcomes have the potential to benefit conservation by improving germination and seedling survival, exemplified by previous studies (*e.g*., Rasmussen *et al.* 1989, 1990). During the past five years, another use for *in vitro* symbiotic germination has surfaced in North America that links the practice with cross pollination, and is discussed here further as a case study.

CASE STUDY 1: CROSSING EFFECTS ON SEED VIABILITY, GERMINATION, AND PROTOCORM GROWTH OF THE EASTERN PRAIRIE FRINGED ORCHID *(PLATANTHERA LEUCOPHAEA)*. The U.S. Federal threatened eastern prairie fringed orchid, *Platanthera leucophaea* (Nutt.) Lindl., is a perennial, terrestrial species endemic to tallgrass prairie remnants and wetlands of the Midwest, and eastward into bogs and fens (Bowles 1983, Sheviak 1974, Sheviak & Bowles 1986). Historical records indicate the species has suffered a 70% decline largely due to the conversion of its habitat to agriculture. Most of the remaining populations are small and fragmented. Given that the species utilizes a facultative breeding system (outcrossing) facilitated by hawkmoth pollination (Bowles *et al.* 2002), small, fragmented populations a fraction of their former size are suspected of contributing to low genetic diversity and inbreeding depression within existing sites. Individual plants typically flower once and are probably short-lived (<10 years). *In situ*, the species utilizes mycorrhizal fungi assignable to the anamorphic genus *Ceratorhiza* (teleomorphs = *Ceratobasidium*) throughout its life cycle (Zettler *et al.* 2005). Efforts to propagate *P. leucophaea* from seed to a leaf-bearing stage via symbiotic germination have been successful *in vitro* after seeds are pre-treated with cold-moist conditions (stratification) and subsequently inoculated with *Ceratorhiza* strains (Zettler *et al.* 2001, 2005). The development of a reliable protocol to propagate *P. leucophaea* from seed with fungi prompted a study to assess how different modes of pollination affect seed viability, germination, and seedling development. This project, conducted in collaboration with Timothy J. Bell (Chicago State University) and Marlin L. Bowles (The Morton Arboretum), is part of a series of studies aimed at eventually broadening the distribution of the species statewide (Illinois). The objective was to determine whether inbreeding and outbreeding depression (dilution of genes associated with local adaptation; disruption of co-adapted gene complexes) occur in *P. leucophaea*, with the ultimate goal of increasing the number of viable populations throughout its range. Crossing experiments took place during two flowering seasons (2000, 2002). In 2000, plants from three populations, separated by >150 km, were experimentally pollinated by hand. In 2002, the experiment was repeated using populations separated by >300 km. Three types of crosses were carried out: self-pollinated (S), pollen transferred to other plants within the same population (out-crossed within, OW), and pollen transferred to other plants in distant populations (out-crossed between, OB). The resulting seed was collected from mature, yellowing, indehiscent capsules, dried over $CaSO_4$ desiccant, and subjected to cold-moist stratification (6^{o} C) lasting >3 months.

TABLE 1. Examples of endangered, threatened or otherwise uncommon North American orchids cultivated using the symbiotic technique. Terrestrial and epiphytic species are denoted by (t) and (e), respectively.

Species	Notes
Bletia urbana Dressler (t)	Mexican endemic; seedlings reintroduced (Ortega-Larrocea 2005)
Cyrtopodium punctatum (L.) Lindl. (e)	Seedlings reintroduced (S.L. Stewart, unpubl. data)
Dichromanthus aurantiacus (t) (La Llave & Lex.) Sal. & Soto Arenas	Seedlings reintroduced (Rangel Villafranco 2006)
Epidendrum nocturnum Jacquin (e)	Seedlings reintroduced (Zettler *et al.* 2006)
Habenaria macroceratitis Willdenow (t)	Seedlings reintroduced (Poulter *et al*. 2005)
Platanthera holochila (Hbd.) Krzl. (t)	Hawaiian endemic, U.S. Federal Endangered; leaf-bearing seedlings obtained *in vitro*, high seedling mortality *ex vitro* (McDonald *et al.* 2006, L.W. Zettler unpubl. data)
P. integrilabia (Correll) Luer (t)	Seedlings established *ex vitro* (Zettler & McInnis 1992)
P. leucophaea (Nuttall) Lindl. (t)	U.S. Federal Threatened; seedlings reintroduced with poor survival (Zettler *et al.* 2005, L.W. Zettler unpubl. data)
P. praeclara Sheviak & Bowles (t)	U.S. Federal Threatened; leaf-bearing seedlings obtained *in vitro* (Sharma *et al*. 2003)
Spiranthes brevilabris Lindl. (t)	Seedlings reintroduced; anthesis <7 months (Stewart *et al.* 2003)
S. longilabris Lindl. (t)	Seedlings reintroduced (L.W. Zettler & K.A. Piskin unpubl. data)

Seeds were sown *in vitro* on an oat-based medium and inoculated with mycorrhizal fungi (*Ceratorhiza goodyerae-repentis* Constantin & Dufour) previously recovered from *P. leucophaea* tissues. Seed viability in both years was significantly lower for S progeny, but there was no significant difference between OW and OB progeny. Percent seed germination was significantly lower for S, but did not differ between OW and OB. These results, albeit preliminary, indicate that starting a new population of *P. leucophaea* from seed should not be detrimental. Although the effects of outbreeding depression may not appear for several generations, it is reasonable to assume that larger distances (>1000 km) are needed for this to occur.

Although symbiotic germination has been applied mostly to temperate terrestrial orchids like *P. leucophaea*, it may also have practical merit for epiphytic species. Epiphytic orchids have long been suspected of being less dependent on mycorrhizal fungi than their terrestrial counterparts, especially at maturity. This may explain, in part, why these plants are less problematic to cultivate from seed in the absence of mycorrhizal fungi (asymbiotic germination). Nevertheless, it is reasonable to assume that epiphytic orchids utilize fungi to some degree to prompt seedling development *in situ*, and evidence now suggests that fungi also provide these plants with a critical source of free water to resist desiccation on arboreal substrates (Yoder *et al*. 2000). Thus, efforts aimed at epiphytic orchid conservation should take into account the potential significant role(s) of such fungi, and act accordingly. This was the impetus of the second case study summarized below and published recently (Zettler *et al.* 2006).

CASE STUDY 2: PROPAGATION OF AN EPIPHYTIC ORCHID (EPIDENDRUM NOCTURNUM) WITH A MYCORRHIZAL FUNGUS.

The genus *Epidendrum* contains ca. 2000 neotropical species, many of which produce appealing floral displays suitable for horticulture. *Epidendrum nocturnum* is no exception. In south Florida, showy epiphytic orchids like *E. nocturnum* have been targeted by poachers leading to legendary stories that have resulted in best-selling novels and at least one movie

TABLE 2. Examples of North American orchid taxa cultivated to the leaf-bearing stage with mycorrhizal fungus strain UAMH 9824 (*Epulorhiza repens* (Bernard) Moore). Terrestrial and epiphytic species are denoted by (t) and (e), respectively.

Species	Notes
Cyrtopodium punctatum (L.) Lindl. (e)	S.L. Stewart *et al.* unpubl. data
Epidendrum nocturnum Jacquin (e)	Zettler *et al.* 2006
H. macroceratitis Willdenow (t)	Stewart & Zettler 2002, Stewart & Kane 2006a, Poulter *et al*. 2005
H. odontopetala Reichenbach (t)	S.L. Stewart unpubl. data
H. repens Nuttall (t)	Stewart & Zettler 2002
Piperia unaluscensis (Sprengel) Ryd. (t)	S.L. Stewart unpubl. data
Platanthera ciliaris (L.) Lindl. (t)	Hartsock *et al.* 2003
P. holochila (Hbd.) Krzl. (t)	Zettler *et al.* 2005
Spiranthes brevilabris Lindl. (t)	Stewart *et al.* 2003, Stewart & Kane 2006b
S. cernua (L.) Rich. (Florida race) (t)	S. L. Stewart unpubl. data
S. delitescens Sheviak (t)	A.J. Hicks unpubl. data
S. longilabris Lindl. (t)	L.W. Zettler & K.A. Piskin unpubl. data
S. magnicamporum Sheviak (t)	listed as *S. cernua* in Wagoner *et al*. 2002
S. odorata (Nuttall) Lindl. (t)	S.L. Stewart unpubl. data

("Adaptation"). In addition, these plants are also threatened by exotic species, habitat loss, and natural disasters (*e.g.*, Hurricane Wilma in 2005). To facilitate their conservation, a project was initiated at Illinois College to cultivate several noteworthy taxa from south Florida in collaboration with Scott L. Stewart (University of Florida's Plant Restoration, Conservation, and Propagation Biotechnology Program) and Larry Richardson (Florida Panther National Wildlife Refuge). Given the successful application of symbiotic germination to terrestrial orchids in North America, the technique was applied to *E. nocturnum* to determine if epiphytic orchids could also be cultivated with fungi. Seeds were obtained from mature capsules at two locations in Collier Co., Florida (Fakahatchee Strand, Florida Panther NWR), promptly dried over $CaSO_4$ desiccant, and stored at –7 ^{o} C for 1-2 years. Seeds were sown on two types of oat-based media and inoculated with the ubiquitous mycorrhizal fungus, *Epulorhiza repens* (Bernard) Moore. The strain of *E. repens* (UAMH 9824; Sbrev-266) originated from Florida where it was isolated from the roots of a terrestrial orchid, *Spiranthes brevilabris* Lindley (Stewart et al., 2003). This fungus was chosen because of its marked ability to prompt seedling development in numerous other taxa (Table 2).

Seed germination commenced within 21 days of sowing and inoculation.

Significant differences in germination were detected between the two seed sources. After 48 days *in vitro*, leaf-bearing seeds were transferred to greenhouse conditions *ex vitro* and placed on pre-sterilized *Sphagnum* moss soaked with or without half-strength commercial fertilizer. After 163 days *ex vitro*, higher seedling survivorship (>90%) occurred on *Sphagnum* lacking the fertilizer. Seedlings originating from a nutrient medium (modified oats medium, MOM; Clements et al., 1986) experienced much higher survivorship on *Sphagnum* containing fertilizer (86%) than seedlings arising from a medium lacking nutrients (44%). Thus, seedlings exposed to nutrients early in their development may have acclimated to the commercial fertilizer *ex vitro*. Pelotons were observed in roots of selected seedlings suggesting that they had a mycotrophic capability, but were infrequent. Seedlings were reintroduced into the Florida Panther NWR 16 months after sowing. Few of

the seedlings survived *in situ* after one year. Efforts are underway to increase survivorship following reintroduction by timing their release with the onset of the rainy season. Although it appears that symbiotic germination may have practical merit for the conservation of *E. nocturnum* and possibly other rare epiphytic orchids, care should be exercised when selecting fungi for this purpose. The fungus species utilized in this study (*E. repens*) is considered a common associate of orchids worldwide, but this particular strain (UAMH 9824) may not be because it was isolated in the northern part of the state (Levy Co., Florida). The decision to use this strain was based on its general geographic origin (Florida), but future, similar studies should utilize fungi from the same habitat if available. Efforts to recover fungi from wild populations of *E. nocturnum* in south Florida have, so far, been unsuccessful.

Recently (August, 2006) efforts have been underway to cultivate at least four other rare orchids from south Florida using both symbiotic and asymbiotic germination: *Epidendrum amphistomum* A. Richard, *E. rigidum* Jacquin, *Polystachya concreta* (Jacquin) Garay & Sweet and *Vanilla phaeantha* Reichenbach. Thus far, *E. amphistomum* and *P. concreta* have been cultured to the leaf-bearing stage *in vitro* on asymbiotic media alone. Seeds of *V. phaeantha* have resisted all treatments and media – perhaps typical for the genus. In nature, *Vanilla* capsules may be consumed and seeds dispersed by invertebrates and vertebrates alike, and it is conceivable that seeds of this genus are problematic to work with because they require an unusual set of pretreatment conditions. Consequently, experiments are being carried out to mechanically scarify *V. phaeantha* seeds using the feeding mechanism (mandibles) of the giant Madagascar hissing cockroach (*Gromphadorhina portentosa* Schaum). Seeds have been fed to these insects in the laboratory, and have been recovered intact from roach frass. An experiment is now being carried out to sow these seeds on artificial media, with and without fungi.

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LITERATURE CITED

Batty, A.L., M.C. Brundrett, K.W. Dixon, & K. Sivasithamparam. 2006. New methods to improve symbiotic propagation of temperate terrestrial orchid seedlings from axenic culture to soil. Austral. J. Bot. 54: 367-374.

Batty, A.L., M.C. Brundrett, K.W. Dixon & K. Sivasithamparam. 2006. *In situ* symbiotic seed germination and propagation of terrestrial orchid seedlings for establishment at field sites. Austral. J. of Bot. 54 : 375-381.

Bowles, M.L. 1983. The tallgrass prairie orchids *Platanthera leucophaea* (Nutt.) Lindl. and *Cypripedium candidum* Muhl. Ex Willd.: some aspects of their status, biology, and ecology, and implications toward management. Nat. Areas J. 3(4) : 14-37.

Bowles, M.L. & T.J. Bell. 1999. Recovery strategies and delisting criteria for *Platanthera leucophaea*, *Asclepias meadii*, *Lespedeza leptostachya*, *Dalea foliosa*, and *Cirsium pitcheri*. Report to the Illinois Endangered Species Protection Board, The Morton Arboretum, Lisle, IL.

Bowles, M.L., K.A. Jacobs, L.W. Zettler & T. Wilson Delaney. 2002. Crossing effects on seed viability and experimental germination of the Federal threatened *Platanthera leucophaea* (Orchidaceae). Rhodora 104 : 14-30.

Clements, M.A., H. Muir & P.J. Cribb. 1986. A preliminary report on the symbiotic germination of European terrestrial orchids. Kew Bull. 41 : 437-445.

Currah, R.S., C.D. Zelmer, S. Hambleton & K.A. Richardson. Fungi from orchid mycorrhizas. In: J. Arditti and A.M. Pridgeon (eds.) Orchid biology: reviews and perspectives, VII (pp. 117-170).

Hartsock, J.J., K.A. Piskin & L.W. Zettler. 2003. Symbiotic seed germination of two showy terrestrial orchids (*Platanthera ciliaris*, *P. cristata*; Orchidaceae) using mycorrhizal fungi. SE Biol. 50 : 149 (abstract).

Ortega-Larrocea, M.P. 2005. Symbiotic propagation and reintroduction of terrestrial orchids in Mexico. Selbyana 26 (1,2) : 361 (abstract).

McDonald, K., S. Hopkins, S. Perlman, & L. Zettler. 2006. The status and propagation of the Federally endangered Hawaiian endemic, *Platanthera holochila* (Orchidaceae). SE Biol. 53 : 209 (abstract).

Poulter, S.B., K.I. McDonald, S.E. Hopkins & L.W. Zettler. Seed propagation of rare epiphytic and terrestrial orchids native to Florida (*Cyrtopodium*, *Epidendrum*, *Habenaria*) using a mycorrhizal fungus. 17th Annual Undergraduate Conference, Butler University, Indianapolis, IN, published abstracts (p. 31).

Rangel Villafranco, M. 2006. Germinación simbiótica y reintroducción de orquídeas terrestres en la Reserva Ecológica del Pedregal de San Ángel, México, D.F. Maestra en Ciencias Biologicas, Universidad Nacional Autonoma de Mexico (UNAM).

Rasmussen, H.N., T.F. Andersen & B. Johansen. 1990. Light stimulation and darkness requirement for the symbiotic germination of *Dactylorhiza majalis in vitro*. Physiol. Plant. 79 : 226-230.

Rasmussen, H.N., B. Johansen & T.F. Andersen. 1989. Density-dependent interactions between seedlings of *Dactylorhiza majalis* (Orchidaceae) in symbiotic *in vitro* culture. Physiol. Plant. 77 : 473-478.

Sharma, J., L.W. Zettler, J.W. Van Sambeek, M. Ellerseck & C.J. Starbuck. 2003. Symbiotic seed germination and mycorrhizae of Federally-threatened *Platanthera praeclara* (Orchidaceae). Am. Mid. Nat. 149 : 79-95.

Shefferson, R.P., M. Weiss, T. Kull & D.L. Taylor. 2005. High specificity generally characterizes mycorrhizal association in rare lady's slipper orchids, genus *Cypripedium*. Mol. Ecol. 14 : 613-626.

Sheviak, C.J. 1974. An introduction to the ecology of the Illinois Orchidaceae. Illinois State Museum Scientific Papers XIV, Springfield, Il.

Sheviak, C.J. & M.L. Bowles. 1986. The prairie fringed orchids: a pollinator-isolated species pair. Rhodora 88 : 267-290.

Stewart, S.L. 2003. The successful reintroduction of the short-lipped ladies'-tresses to Florida, USA: implications for the future of native orchid restoration. In: Soorae P.S. (ed.) Reintroduction NEWS, No. 22, Newsletter of the IUCN/SSC Reintroduction Specialist Group (pp. 21-22).

Stewart, S.L. & M.E. Kane. 2006. Symbiotic seed germination of *Habenaria macroceratitis* (Orchidaceae), a rare Florida terrestrial orchid. Plant Cell Tiss. Organ Cult. 86 : 159-167.

Stewart, S.L. & M.E. Kane. 2006. *In vitro* specificity exhibited by fungal mycobionts of *Spiranthes floridana* (Orchidaceae) within the congener *Spiranthes brevilabris*. *In Vitro* 42:37-A (abstract).

Stewart, S.L. & L.W. Zettler. 2002. Symbiotic germination of three semi-aquatic rein orchids (*Habenaria macroceratitis*, *H. quinqueseta*, *H. repens*) from Florida. Aquatic Bot. 72 : 25-35.

Stewart, S.L., L.W. Zettler, J. Minso & P.M. Brown. 2003. Symbiotic germination and reintroduction of *Spiranthes brevilabris* Lindley, and endangered orchid native to Florida. Selbyana 24 : 64-70.

Wagoner, C.S., A.L. Stice, S.L. Stewart & L.W. Zettler. 2002. Seed propagation of *Spiranthes cernua* (L.) Rich. (Orchidaceae) using mycorrhizal fungi from two genera (*Epulorhiza* and *Ceratorhiza*). SE Biol. 49 : 168 (abstract).

Yoder, J.A., L.W. Zettler & S.L. Stewart. 2000. Water requirements of terrestrial and epiphytic orchid seeds and seedlings, and evidence for water uptake by means of mycotrophy. Plant Sci. 156 : 145-150.

Zettler, L.W. Nature's fungus connoisseurs: new insight into the mysterious orchid-fungal association. Orchids 74(4) : 292-297.

Zettler, L.W. & T.M. McInnis, Jr. 1992. Propagation of *Platanthera integrilabia* (Correll) Luer, and endangered terrestrial orchid, through symbiotic seed germination. Lindleyana 7 : 154-161.

Zettler, L.W., S. Perlman, D.J. Dennis, S.E. Hopkins & S.B. Poulter. 2005. Symbiotic germination of the Federally endangered Hawaiian endemic, *Platanthera holochila* (Orchidaceae) using a mycobiont from Florida: a conservation dilemma. Selbyana 26 (1,2) : 269-276.

Zettler, L.W., K.A. Piskin, S.L. Stewart, J.J. Hartsock, M.L. Bowles & T.J. Bell. 2005. Protocorm mycobionts of a Federally threatened orchid, *Platanthera leucophaea*, and a technique to promote leaf formation in seedlings. Stud. Mycol. 53 : 163-171.

Zettler, L.W., S.B. Poulter, K.I. McDonald & S.L. Stewart. 2006. Conservation-driven propagation of an epiphytic orchid (*Epidendrum nocturnum*) with a mycorrhizal fungus. HortSci. 42(1) : (in press)

Zettler, L.W., S.L. Stewart, M.L. Bowles & K.A. Jacobs. 2001. Mycorrhizal fungi and cold-assisted symbiotic germination of the Federally threatened eastern prairie fringed orchid, *Platanthera leucophaea* (Nuttall) Lindley. Am. Mid. Nat. 145 : 168-175.

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SEM AND PCR STUDY OF MYCORRHIZAL FUNGI ISOLATED FROM THE AUSTRALIAN TERRESTRIAL ORCHID: *PRASOPHYLLUM*

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Most members of the genus *Prasophyllum* (Leek Orchids) are threatened and restricted in distribution in Australia. *Prasophyllum* species are obligate mycotrophic plants and current conservation protocols for terrestrial orchids in Australia require propagation with symbiotic mycorrhizal fungi. Unfortunately there is a paucity of knowledge regarding the mycosymbiont in this genus, hampering conservation and re-introduction efforts.

Before recovery plans can be implemented for *Prasophyllum* basic biological information is required about the nature of the mycorrhizal relationship. This study used two threatened *Prasophyllum* species: *P.* sp. aff. *validum* and *P. diversiflorum*, both from south-west Victoria. *Prasophyllum* sp. aff. *validum* grows in a low open grassy heathland and *Prasophyllum diversiflorum* (Gorae Leek Orchid) is found in open grassy, swampy vegetation. Underground plant parts were collected for mycorrhizal isolation and Scanning Electron Microscopy (SEM) studies. Mycorrhizal fungi were isolated from adult plants at four times during the year: soon after leaves appeared following summer, during the period of flower bud growth (Winter), while flowering (Spring) and as the fruit developed (Spring) SEM was used to determine the location, type and amount of mycorrhizal colonisation. The ability for the isolated fungi to germinate seed was tested with seed collected from plants in 2005. As most mycorrhizal fungi from Australian terrestrial orchids do not sporulate in culture and therefore cannot be identified by normal taxonomic means DNA from fungal isolates were ITS-sequenced and closest GenBank matches were determined. The information gained in this study will provide the basis for further re-introduction and conservation studies.

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DESARROLLO DE CAPSULAS Y GERMINACION *IN VITRO* DE *PHRAGMIPEDIUM HUMBOLDTII, P. LONGIFOLIUM* Y *P. PEARCEI*

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ABSTRACT. Slipper orchids belonging to the genus *Phragmipedium* (subfam. Cypripedioideae) are seriously threatened and therefore listed in Appendix I of CITES. Less research has been conducted in this genus than in others belonging to the same subfamily. In this work, we evaluated development of capsules (seed pods) from *Phragmipedium humboldtii, P. longifolium* and *P. peaarcei* from the time of pollination until opening. Moreover, seed viability was tested with the tetrazolium method in each of the capsules that were subsequently used to evaluate the effect of light and two culture media (Knudson C vs. Murashige and Skoog half concentrated) on in vitro asymbiotic germination and seedling growth. 100% of the pollinated flowers developed capsules, which differentiated in terms of length and diameter among species. While the length of the capsules remained constant during development, their diameter increased during the first 6-8 weeks and then stopped. Time required for maturity and opening of the capsules also varied among species (31 weeks in *P. humboldtii*, 16 weeks in *P. longifolium* and 9.5 weeks in *P. percei*). Seed viability differed among species as well, averaging 34.3% in *P. humboldtii*, 44.7% in *P. longifolium* and 82.3% in *P. pearcei*. Furthermore, seed viability of each capsule was used to adjust the germination rate measured in each case. While very few *P. humboldtii* viable seeds germinated under the conditions tested (2.9%), better results were observed in the other two species (close to 40% germination). No significant effect of light/darkness regime or of culture medium was observed on germination. However, better growth of the germinated embryos was observed with the Knudson C medium and darkness conditions. Further subculture of the growing plantlets under light conditions induced development of roots and allowed successful acclimatization of seedlings in the greenhouse.

PALABRAS CLAVE: *Phragmipedium,* orquídeas terrestres, polinización, desarrollo de cápsulas, cloruro de tetrazolio, germinación *in vitro*.

Las orquídeas del género *Phragmipedium* Rolfe (subfamilia Cypripedioideae), comúnmente llamadas zapatillas o "slipper orchids", se encuentran naturalmente distribuidas en Meso y Suramérica (Cox *et al.* 1998, Dressler 2003). Estas plantas están en peligro de extinción por alteración o destrucción de su hábitat y por la extracción masiva de plantas de su ambiente natural (Arditti 1992, Salazar 1996).

El hecho de que el género *Phragmipedium* haya sido relativamente poco estudiado probablemente se debe al hecho de que si bien algunas especies producen flores vistosas, su cultivo no es fácil, lo cual ha limitado su atractivo para coleccionistas y viveristas. Este estudio pretende describir el desarrollo y determinar el tiempo de maduración de cápsulas de *P. humboldtii, P. longifolium* y *P. pearcei*, así como evaluar la viabilidad de las semillas en las mismas, y establecer un método para la germinación *in vitro* de semillas maduras de estas tres especies y el desarrollo posterior de plántulas.

Materiales y métodos

Se polinizaron manualmente flores de plantas de *Phragmipedium humboldtii*, de *P. longifolium* y de *P. pearcei*, en cultivo en el Jardín Botánico Lankester de la Universidad de Costa Rica. Cada semana, a partir de la fecha de polinización, se midió el largo y el diámetro de cada cápsula. Además, se determinó el tiempo transcurrido para la apertura de cada cápsula. Parte de las semillas de las cápsulas abiertas de las plantas mencionadas anteriormente se utilizaron para medir el porcentaje de viabilidad mediante el método

de tinción con cloruro de tetrazolio (Singh 1981). Se desinfectaron las semillas restantes en cada cápsula mediante agitación durante 10 min en hipoclorito de sodio (0.6%) adicionado con Tween 80 (1 gota/100 ml), seguida de tres lavados con agua destilada estéril. Las semillas se colocaron en placas de Petri de 90 mm de diámetro con 20 ml de medio de cultivo semisólido. Para la germinación *in vitro,* se evaluaron los medios de cultivo Knudson C (Knudson 1946) y Murashige y Skoog (1962) utilizando, en este último, las concentraciones de macro y micronutrientes al 50% (MS 50%). Ambos medios fueron complementados con 1 mg/l de tiamina, ácido nicotínico y piridoxina y 20 g/l de sacarosa. El pH se ajustó a 5.7 y los medios fueron gelificados con 0.8 % de agar. La temperatura de cultivo fue 25±1°C. Se evaluó la germinación en oscuridad y en un fotoperíodo de 12 h (10.9 µmol m^{-2} s^{-1}, Sylvania Supersaver Cool White, 32 W, F48T12/CW/SS).

Para determinar el porcentaje de germinación, en cada placa se marcaron tres áreas de aproximadamente 1 cm^2. Se cuantificó el número inicial de semillas en cada área el día en que se cultivaron. En cada evaluación se contó el número de semillas germinadas. Se utilizaron tres placas de Petri para cada una de las combinaciones de tratamientos de medios de cultivo y régimen lumínico por especie. El porcentaje de germinación se corrigió utilizando el porcentaje de viabilidad de cada cápsula.

Las plantas obtenidas fueron aclimatadas en recipientes plásticos (3 x 3 x 4 cm) con turba picada. Se utilizó riego por nebulización (4 s cada 15 min durante el día).

Se realizó un análisis de varianza (ANDEVA) y se utilizó la prueba de Tukey para comparación de medias (Statistica, StatSoft, Tulsa, OK, EUA) para determinar diferencias entre especies y tratamientos.

Resultados

Todas las flores polinizadas formaron cápsula. Las cápsulas más grandes fueron las de *P. humboldtii*, cuyo tamaño promedio, una semana antes de la apertura, fue 182,7±3,2 mm x 7.0±0.2 mm, seguidas por las de *P. longifolium* (60.02±2.0 mm x 5.6±0.1 mm), y las cápsulas más pequeñas fueron la de *P. pearcei* (42.5±1.4 mm x 4.2±0.1 mm) Las cápsulas de *P. humboldtii* fueron las que tardaron más tiempo en abrir (31 semanas), mientras que las de *P. longifolium* y las de *P. pearcei* duraron un promedio de 16 y 9.5 semanas, respectivamente.

La viabilidad promedio de las semillas fue de 34.3% en las cápsulas de *P. humboldtii*, de 44.7% en *P. longifolium* y de 82.3% en *P. pearcei*. El porcentaje de viabilidad de cada cápsula se utilizó para corregir el porcentaje de germinación en cada caso. Este porcentaje de germinación corregido fue menor en *P. humboldtii* (2.9%) que en *P. longifolium* (41.3%) y en *P. pearcei* (38.7%), sin que hubiera diferencias significativas entre estos dos últimos.

Ni el medio de cultivo ni el régimen lumínico utilizados tuvieron efecto sobre la germinación de las especies estudiadas ($p>0.05$). Sí se observó un mejor crecimiento de los protocormos de *P. pearcei* y de *P. longifolium* en el medio Knudson C que en el MS 50%. Además, dentro de los cultivados en Knudson C, se observó un mejor crecimiento de los protocormos germinados en la oscuridad. Estos se pasaron a fotoperíodo de 12 horas después de 6 semanas de cultivo, y una semana después empezaron a cambiar su coloración de blanco a verde.

Los protocormos de *P. pearcei* y *P. longifolium* empezaron a desarrollar la primera hoja a las cuatro semanas después de la siembra de las semillas, mientras que en *P. humboldtii* esta se empezó a desarrollar en la semana ocho de cultivo. Después de tres meses de cultivo, las plántulas de *P. pearcei* y *P. longifolium* tenían dos o más hojas y empezaron a desarrollar raíces a los tres meses y medio. Se logró aclimatar con éxito todas las plantas transferidas al invernadero, siguiendo la metodología descrita anteriormente.

Conclusiones

En este estudio se describieron las curvas de crecimiento y se determinó el tiempo que necesitan las cápsulas de *P. humboldtii, P. longifolium* y *P. pearcei* para llegar a madurez y abrirse. Esto último es muy útil para determinar el momento adecuado para la recolecta de cápsulas inmaduras, las cuales son más fáciles de desinfectar en estas condiciones. Además, en este trabajo se describe un método efectivo para la germinación y el desarrollo de plántulas de las tres especies de *Phragmipedium* estudiadas.

Mediante este procedimiento se puede multiplicar gran cantidad de plantas de este género, manteniendo una variabilidad genética mayor a la obtenida mediante la propagación clonal. Esto es muy ventajoso para la producción de plantas con fines de conservación de la especie.

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LITERATURA CITADA

Arditti, J. 1992. Fundaments of Orchid Biology. John Wiley & Sons, Inc., EUA. 691p.

Cox, A.V., A.M. Pridgeon & M.W. Chase. 1998. The utility of DNA sequence data in orchid systematics: an example from the slipper orchids (Cypripedioideae), pp. 265-273. *In*: C. E. B. Pereira (ed.). Proceedings of 15th World Orchid Conference. Naturalia, Francia.

Dressler, R.L. 2003. Orchidaceae, pp. 1-595. *In:* Hammel, B.E., Grayum, M.H., Herrera, C. & Zamora, N. (eds.). Manual de Plantas de Costa Rica, vol III: Monocotiledóneas (Orchidaceae-Zingiberaceae). Missouri Botanical Garden. San Luis, Misuri.

Knudson, L. 1946. A new nutrient solution for the germination of orchid seed. Amer. Orchid Soc. Bull. 15:214-217.

Murashige, T. & F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-497.

Salazar, G.A. 1996. Conservation Threats, pp. 6-10. *In:* Hágsater, E & V. Dumont (eds.). Orchids- Status Survey and Conservation Action Plan. IUCN. Gland Switzerland and Cambridge, UK.

Singh, F. 1981. Differential staining of orchids seeds for viability testing. Amer. Orchid Soc. Bull. 50: 416-418.

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ECOLOGY OF ORCHIDS IN URBAN BUSHLAND RESERVES – CAN ORCHIDS BE USED AS INDICATORS OF VEGETATION CONDITION?

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KEY WORDS: terrestrial orchid, indicators, urban reserves, vegetation condition, mycorrhiza, pollination

The loss of urban native vegetation is a global crisis particularly as cities continue to expand and populations grow. Native vegetation often remains as small isolated fragments embedded in the human matrix of urban development. These remnants become islands of biodiversity that experience varying degrees of degradation due to their high perimeter to area ratio. Habitat loss in the biodiversity hotspot of south west Western Australia is considered to be one of the major threats to native terrestrial orchids, and is in part responsible for the current listing of 35 species as critically endangered (Western Australian Government 2006). Diminishing habitats and fragmentation of populations by urban development raises questions as to the sustainability of remnant populations. However, despite the loss of habitat, orchids continue to persist in the urban environment although little is known in detail of their ecological response to such pressures. Orchids are a highly specialized group of plants, their pollination methods and mycorrhizal associations ensure complex interactions with their environment. Do these interactions provide a measurable way to assess the health of ecosystems? Six orchid species of varying pollination mechanisms, associated mycorrhiza and growth form are used in this study. Although the study species are common to the Swan Coastal Plain, many are congeners to much rarer species currently under threat of extinction. This study provides insights into orchid ecology by examining pollination rates, mycorrhizal relationships and field plant establishment. Measurement and quantification of ecological responses of orchids in reference to varying site condition variables aims to determine whether orchids can be successfully utilized as indicators of vegetation condition.

Vegetation Condition Assessment

There are many disturbances that originate from the surrounding urban matrix including fire, nutrient enrichment and weed invasion. Disturbance in urban bushland remnants impacts upon the general condition and sustainability of the vegetation community. The structure and function of the urban remnant can become compromised and the vegetation may undergo change, the extent of which will be determined by the underlying vigour, resilience and organization of the vegetation (Calow 1995, Hobbs 2001). Various methods of vegetation condition assessment were compared in this study to determine which method may be most suitable for application to urban bushland reserves. The methods investigated include The Habitat Hectares method (Parkes *et al.* 2003). This method utilizes a benchmark condition against which the condition of other sites is measured. The Viability Estimate developed by Del Marco *et al.* (2004), which is designed to be used by local government, land managers and trained volunteers, but not necessarily botanical ecologists. It provides a more qualitative determination of vegetation condition based on visual estimation. The final method explored is the Vegetation Condition Index (VCI), developed by Stenhouse (2005) as a quantita-

tive tool for investigating the effects of anthropogenic disturbance on vegetation condition. The presence of orchids is compared with site rankings based on vegetation condition to determine if there is a relationship between orchid presence and vegetation condition. Further interrogation of the data is carried out to ascertain whether certain orchid species were potential indicator species.

Pollination

As bushland fragments become more isolated, visitation by pollinators, plant fecundity and population survival diminishes (Hobbs & Yates 2003). Orchids are dependent on the presence of pollinators for their continued survival. Some orchids are more specific in the cadre of pollinators that repeatedly and successfully provide pollination success. Orchids may provide a pollinator reward, or as is more common in the south west Australian orchids, be a floral deception. Other orchid species are more generalist relying on pollination by foragers or incidental events (Dafni & Bernhardt 1990). Due to this dependence on pollinators, orchids may be used to provide information on insect communities in the area. Measuring pollination success, by seed set, provides an indication of the general health and condition of the site. This study looked at pollination success over two consecutive years using both hand and natural pollination in order to measure pollination as a response to varying vegetation conditions. Experiments were also conducted to determine if resource limitation was occurring as a result of poor vegetation condition.

Mycorrhiza

Orchids rely on their mycorrhizal associations in order to germinate and in many species this dependency carries on into adulthood with seasonal reinfection (Rasmussen 2002). Orchids range from the very specific to the more general in relation to the mycorrhiza they play host to. The persistence of the correct fungus species is required for the survival of the orchids. However, soil nutrients, disturbance, soil moisture and litter layer may all influence the distribution of orchid mycorrhiza. Measuring the distribution and presence of the fungi provides insights to the general health and condition of the site. Seven orchid species were used in baiting experiments to determine the potential extent of fungal distribution using *ex situ* methods and ecological distribution using *in situ* methods. Correlations with microhabitat and broader scale condition data show potential relationships between habitat and mycorrhizal distribution. Distribution of mycorrhiza in relation to the adult host plant is investigated through an *in situ* baiting experiment. The identification of many of the mycorrhiza recovered from adult hosts across sites of varying condition is made using the primers ITS1F and ITSR. Genetic sequencing of mycorrhizal isolates allows a thorough investigation of fungal specificity and the influence of environmental conditions on fungal presence.

The complex interactions of orchids with their environment mean they are sensitive to environmental changes. Information may be gained on the general health and condition of the ecosystem by measuring the health and responses of orchid populations, as this study investigates. However, if the use of orchids as indicators proves to be inappropriate, the knowledge on orchid ecology gained from such studies may be invaluable in future planning and management of urban reserves.

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LITERATURE CITED

Calow, P. 1995. *In*: D. Rapport, C. Gaudet & P. Calow (eds.). Evaluating and Monitoring the Health of Large-Scale Ecosystems. Springer – Verlag, Germany.

Dafni, A., & P. Bernhardt. 1990. Pollination of terrestrial orchids of southern Australia and the Mediterranean region. Evol. Biol. 24: 193-253.

Del Marco, A., R. Taylor, K. Clarke, K. Savage, J. Cullity, & C. Miles. 2004. Local Government Biodiversity Planning Guidelines for the Perth Metropolitan Region. Western Australian Local Government Association, Perth.

Hobbs, R. 2001. Synergisms among habitat fragmentation, livestock grazing and biotic invasions in south-western

Australia. *Conservation Biology* 15: 1522-1528.

Hobbs, R., & C. Yates. 2003. Impacts of ecosystem fragmentation on plant populations: generalizing the idiosyncratic. Austral. J. Bot. 51**:** 471-488.

Parkes, D., G. Newell & D. Cheal. 2003. Asessing the quality of native vegetation: the 'habitat hectares' approach. Ecol. Manag. Restor. 4**:** 29-38.

Rasmussen, H. 2002. Recent developments in the study of orchid mycorrhiza. *Pl. Soil* 244: 149-163.

Stenhouse, R. 2005. Ecology and Management of Bushland in Australian Cities. PhD thesis, University of Western Australia.

Western Australian Government. 2006. Wildlife Conservation (Rare Flora) Notice 2006(2). Western Australian Government Gazette 2006. Perth, Western Australia.

Belinda Newman was educated at Murdoch University, Perth, Australia, where she received a Bachelor of Environmental Science. She is currently completing a Ph.D. in environmental science from Murdoch University, looking at the ecology of orchids in urban bushland remnants and their use as indicators of vegetation condition. Belinda's research interests include vegetation condition and ecosystem health, effects of disturbance on orchid populations and orchid pollination.

Phil Ladd is a senior lecturer in ecology at Murdoch University, Perth, Australia. He is particularly interested in pollination ecology, community ecology and population biology. Phil is also interested in all aspects of palaeobotany including palynology and past vegetation history and the reconstruction of paleaoclimates based on vegetation reconstruction. Current research involves pollination ecology in buzz pollinated Western Australian natives and the conservation of the rare plant *Lambertia orbifolia.*

Andrew Batty recieved his Ph.D in 2001 specializing in the role of symbiotic seed germination and seed ecology in the conservation of selected Western Australian terrestrial orchids. His research interests include conservation biology of rare orchids and he has worked on the Millenium Seed Bank Project.

Kingsley Dixon has over 20 years experience in researching the ecology and physiology of Australian native plants and ecosystems. He leads a science group comprising botanical and restoration sciences and, as Director of Science at the Botanic Gardens and Parks Authority (BGPA), has developed a strong multi-disciplinary approach to conservation and restoration of native plant biodiversity and degraded landscapes. This research group has contributed significantly to seed science in Australia, with major advances in understanding seed dormancy as well as orchid seed conservation.

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CORES PROJECT - CONSERVATION OF ENDANGERED ORCHIDS: AN ACTION PLAN FOR CONSERVATION OF BRAZILIAN ORCHIDS

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The Cores Project, intent to collect biological and antropic informations on nine species of orchids that are included on the official list of threatened species of Brazil (*Cattleya schilleriana*, *Laelia fidelensis*, *L. lobata*, *L. grandis*, *L. jongheana*, *L. virens*, *L. perrini*, *L. tenebrosa* and *L. xanthina*) aiming to revaluate its conservation status and elaborate an action plan to each one in particular.

This project is being executed at the Rio the Janeiro Botanical Garden Research Institute (JBRJ) and the Leopoldo Américo M. de Mello Development and Research Institute (CENPES/PETROBRAS), counting with two teams, one in Rio de Janeiro and the other in Espírito Santo, of 15 researchers altogether. It has three years length, starting from January 2006.

Due to the fact that the distribution of these species concentrates on the Atlantic Forest, specially on the southeast region, a Hotspot with less then 10% of its original ranging, it becomes essential to study the natural populations as well as *ex situ* conservation techniques. Some of the species present a distribution restrict to some regions of Espírito Santo (*C. schilleriana*, *L. tenebrosa*, *L. xanthina*), Minas Gerais (*L. jongheana*) and Rio de Janeiro (*L. lobata*, *L. fidelensis*), while *L. grandis* can be found in areas of two states, south Bahia and north Espírito Santo, but not less restrict then the others mentioned before. *L. perrini*, *L. virens* present wider distribution in Espírito Santo, Minas Gerais, Rio de Janeiro and São Paulo states.

To elaborate the action plan for these species, revaluating its conservation status, qualitative and quantitative data of the natural populations are being studied, identifying the geographic distribution patterns, investigating the reproductive biology characteristics and elaborating semi-structured interviwes, that takes place on orchids exhibitions, intending to get in contact with a part of the population that takes part directly on the conservation of threatned species (as orchid lovers, cultivators and collectors). As part of the *ex situ* conservation plan, DNA samples are being stored at the JBRJ germoplasm bank and at the CENPES Orchidarium.Mesoamerica, Caribbean and Mexico. Twenty two main areas were identified using complementarity analysis and considered as prioritaire for the conservation of total diversity of *Bulbophyllum* in the Neotropics. Some of this areas are located in previously proposed Hotspots for other plant and animal groups in the American continent.

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FUNGUS-ASSISTED REINTRODUCTION AND LONG-TERM SURVIVAL OF TWO MEXICAN TERRESTRIAL ORCHIDS IN THE NATURAL HABITAT

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Preservation of genetic diversity of orchids for conservation and restoration purposes is now a feasible practice after the ecological studies of seed bank dynamics made by several investigators (Batty *et al.* 2001, Whigham *et al.* 2006). However, few studies have demonstrated the reliability of reintroduction of several species into their natural habitat and less, managing symbiotic fungus (Ramsay and Dixon 2003, Zettler *et al.* 2003). The main problem is the survival monitoring of reintroduced populations because of the long length of the project and the results depend on the species, pollinators and habitat characteristics (Ramsay and Stewart 1998, Stewart *et al.* 2003, Ramsay and Dixon 2003). In consequence, data obtained after several years can not be conclusive for the establishment of reliable protocols to recover endangered populations. Schuiteman and Vogel (2003) enlist several aspects that must be considered when dealing real practical problems to conserve orchids. The selection of target species is one of these considerations and *in situ* as well as *ex situ* strategies could be made sometimes together (Brundrett *et al.* 2003). The selection of species for managed conservation processes can be mainly driven by two forces: the degree of endanger and the feasibility of propagation (Feuerherdt *et al.* 2005).

Because of working with endangered species in a particular habitat is not always possible, model species can also be selected such as those orchids that are well adapted to environmental changes and which seed bank is always easy to acquire. This can lead us to obtain useful information about efficiency of isolated fungus and also alternatives to manage attractive species that can be offered to botanical gardens. At the same time, this strategy can get the attention of amateurs and collectors without detrimental endangered populations and provide founds for financing long-term monitoring projects.

Since 2001, with the help of students, we started a project for symbiotic propagation of terrestrial orchids from southern Mexico City (see Rangel-Villafranco & Ortega-Larrocea in the same volume). Two species of Mexico have been selected for starting monitoring reintroduction of populations. Seedlings were cultivated *in vitro* with mycorrhizal fungi and successfully reintroduced within a biological reserve located in Mexico City. *Bletia urbana* Dressler, is an endangered species with a infrequently seed production and very scarce seed bank collection and has been long-term documented (Ortega-Larrocea 2004). Despite the fact that natural populations are annually growing during the rainy season, after some years of monitoring we have realized that flowering is not produced each year and in consequence, seeds are not easy to get. Acquisition of capsules was done for the first time in 1984 (Martínez 1985, Rubluo *et al.* 1989) and for a second time in 2000. We have demonstrated that seeds of this species can be preserved without detrimental in their germination for more than 16 years only in a refrigerator (Ortega-Larrocea *et al.* 2000). Seedlings of *B. urbana* coming from both seed banks were introduced coinciding with the rainy season to promote survival (Castillo 2002). We have monitored growth for the past six years with a final survival of *ca.* 50 %. Means of plant sizes revealed that *B. urbana* requires several years to reach adulthood size. After five years, plants start to flower and seeds were subsequently collected to assess embryo viability (Fig.1A). More than 90 % of seeds produced naturally by the reintroduced plant germinated *in vitro* (Fig. 2A). However, the continuity of this process has also been affected by natural conditions as high and prolonged precipitation, affect-

FIGURE 1. *Bletia urbana.* **A.** Flowering in natural conditions of symbiotic reintroduced seedlings. **B.** Native adult plant showing a lot of capsules. **C.** *Dichromanthus aurantiacus* plantlets emerging after two years of survival under natural conditions. **D.** Re-emerging plant after one year of reintroduction. **E.** Re-emerging plants after one year of survival into its habitat.

ing seed viability (Fig. 2B). Intact seeds that were not damaged while pathogen atack germinated as usual and we have demonstrated that self-propagation mechanism for this species has been reached naturally. The complete monitoring of this particular species will be published soon (Ortega-Larrocea *et al.*, submitted).

We conducted further experiments to determine whether the seeds produced in 2005 could also germinate under natural conditions the following year (June 2006). Seeds baits were placed *in situ* in the vicinity of the adult plants and closely monitored. Preliminary results demonstrate that recovering protocorms in a short time is not feasible and most of the seeds that have been monitored remain with no germination after months (Fig. 2C).

The second species that we selected was a widespread and abundant terrestrial orchid *Dichromanthus aurantiacus*(La llave & Lexarza) Salazar & Soto Arenas. It seems that habitat perturbation favored the dispersion of this species and can currently be observed long distributed, in conserved habitat patches, as well as in road ways (Téllez 2002, Espejo *et al*. 2002, Hágsater *et al.* 2005). Contrary to *B. urbana*, *D. aurantiacus* flower each year and billions of seeds can be collected for many developed capsules (Fig. 1B). This plant, called *cutsis*, is very attractive to collectors and it is used for ornamentation (Hágstater *et al.* 2005). Symbiotic propagation has been done successfully by Rangel (2004) and recently reintroduction into their natural habitat in two successive years (2004 and 2005) (Rangel 2006). Reintroductions were performed into different microsites and have been monitored for the past two years. These orchids have been symbiotically propagated with fungi isolated from adult plants. During the first year (2005), 15 % of survival was recorded for 91 reintroduced seedlings in five microsites. By the second year, less than 5 % of this population re-emerged (Fig.1C). In 2005, a second population of 131 seedlings was propagated with ten different isolates of fungi coming from adult plants as well as from protocorms obtained by seed baiting. Seedlings were reintroduced in 13 microsites within the same habitat and near a quarter of plants survive after a year. Survival and growth parameters indicate that there is not any short-term effect from the origin of the isolate (Rangel and Ortega-Larrocea in prep.). Microsite conditions as high organic matter and light seem to favor survival and play a fundamental role in plant development (1D).

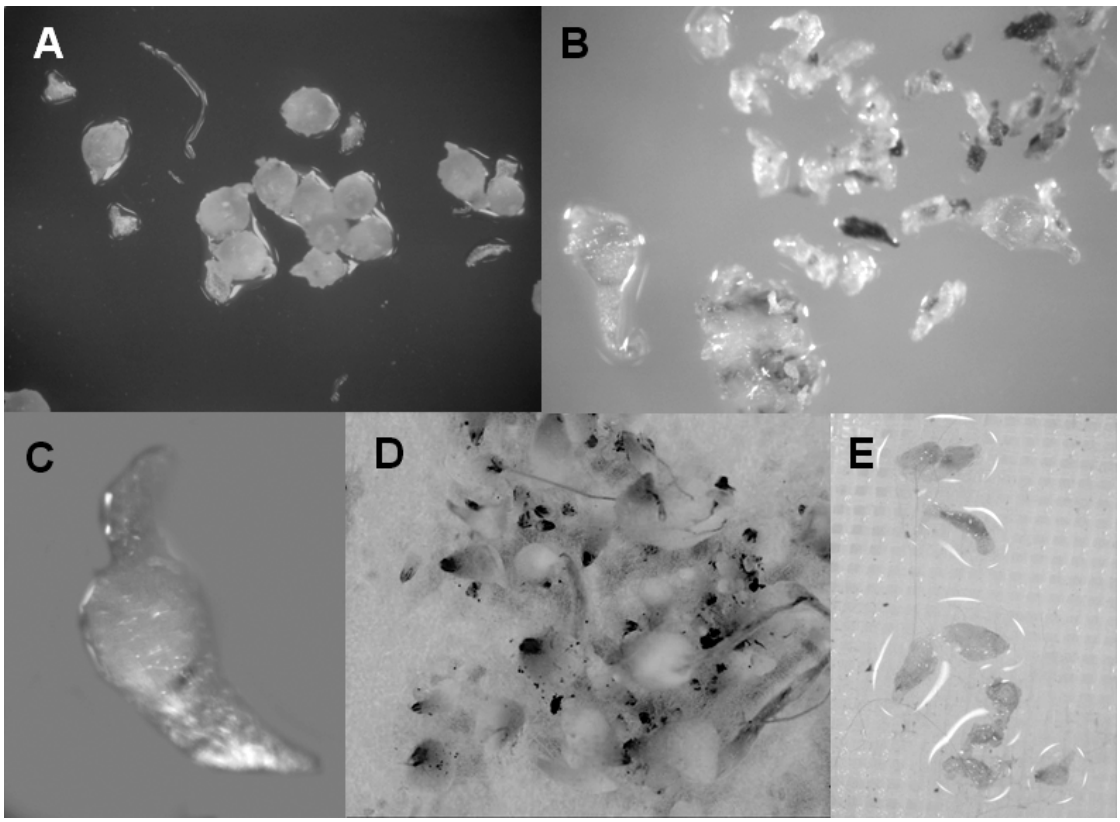

FIGURE 2. *Bletia urbana* **A.** Asymbiotic protocorms of *B. urbana* from a capsule of a reintroduced plant. **B.** Damaged seeds by natural conditions and some rescued *in vitro* protocorms from a reintroduced plant. **C.** embryo recovered *in vitro* after incubation in soil for months and produced by reintroduced plants in nature. *Dichromanthus aurantiacus* **D.** seedlings obtained through baits. **E.** Imbibed seeds of *Bletia campanulata* after several months of incubation in soil.

The mycorrhizal fungi that made symbiotic associations with these two orchids belonged to two genus. Adult plants of *B. urbana* are associated naturally with *Epulorhiza* fungi and *D. aurantiacus* with *Cerathorhiza. In vitro* propagation has shown that *B. urbana* is easy to germinate even asymbiotically (Martínez 1991, Rubluo *et al.* 1993), it is less specific for isolates and can have association also with *Ceratorhiza* fungus (Rangel 2004). *Dichromanthus auranticus* can also be developed with some *Epulorhiza* isolates. However the low compatibility lead to obtain protocorms that do not overcome stage 3 and also asymbiotic germination is difficult to achieve (Rangel 2006). For both species, development of seedlings is faster and better when propagated with an isolate from the same adult species.

In situ germination showed that fungus specificity for *D. aurantiacus* with *Cerathoriza* is not random and isolates of this genus have been obtained when incubated in the rhizosphere of adult *Bletia urbana*. Baiting also reveals that this species can develop easily in few months during the rainy season (Fig. 2D) instead conditions for species of genus *Bletia* are not yet understood (Fig. 2C and E). We have hypothesized that light could be one of the factors that limits *Bletia* spp. germination but asymbiotic protocorms can be obtained *in vitro* when germinate in obscurity. This evidence could support the fact that in natural conditions, *D. aurantiacus* is more wide spread and seedlings can be found near adult populations also because seed production is regular in a lot of capsules. On the other hand, *B. urbana* seedlings have never been observed under natural conditions.

If analyzing the results of survival of reintroduced plants, recovery of *D. aurntiacus* could be less feasible that *B. urbana* because it is very poor and growth of re-emerging plants is not increasing as a measure of adap-

tation: see size of re-emerging *B. urbana* plants after a year compared with those of *D. aurantiacus* after two years (Fig.1C and E). Microsite conditions are influencing much more the survival of reintroduced plants of this wide spread species, contrary to what we can expect for. In contrast, management symbiotic propagation and reintroduction seems to favor the endangered *B. urbana.* This is because their high survival is less dependent on microsite conditions every year, and flowering and self pollination produce capsules with viable seeds. However, these results are preliminary examples of the successful reintroduction in pilot essays in natural conditions and are not conclusive. Further essays with symbiotic seedlings of the same species could not be repetitive, particularly in the case of *B. urbana* because we have not proved several isolates as we do for *D. aurantiacus*.

Meanwhile, we illustrate the fact that even when natural populations of some species are decreasing very fast, management can sometimes easily recover some species in a relatively short time. Also we open the question whether *D. aurantiacus* is so aggressive and widespread: it is because of a synergistic effect of reproduction and association with mycorrhizal partner that promote its survival in nature? Could it be the opposite for the endangered *B. urbana* and a poor seed bank possibly risked survival because fungus soil populations of *Epulorhiza* are less abundant or less infective? Further studies must be conducted in the short future to investigate whether other plant species of these two genus behave similarly. Working with these contrasting orchids in the same habitat lead us to learn important constraints about real management of species for restoration purposes and are quite interesting from the conservation point of view.

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LITERATURE CITED

Batty, A.L., K.W. Dixon, M. Brundrett & K. Sivasithamparam. 2001. Long-term storage of mycorrhizal fungi and seed as a tool for the conservation of endangered Western Australian terrestrial orchids. Aust. J. Bot. 49: 619-628.

Brundrett, M.C., A. Scade, A.L. Baty, K.W. Dixon & K. Sivasithamparam. 2003. Development of *in situ* and *ex situ* seed baiting techniques to detect mycorrhizal fungi from terrestrial orchid habitats. Mycol. Res. 107: 1210-1220.

Castillo, M.M. 2002. Micorrización *in vitro* de *Bletia urbana* como una estrategia para su reintroducción. Tesis de licenciatura, Facultad de Ciencias, Universidad Nacional Autónoma de México. 52 p.

Espejo, S.A., C.J. García, F.A. López, M.R. Jiménez & S.L. Sánchez. 2002. Orquídeas del Estado de Morelos. Orquídea (Méx.) 16: 332 p.

Feuerherdt, L., S. Petit & M. Jusaitis. 2005. Distribution of mycorrhizal fungus associated whit the endangered pink-lipped spider orchid (*Arachnorchis* (syn. *Caladenia*) *behrii*) at Warren conservation park in south Australia. New Zeal. J. Bot. 43: 367-371.

Hágsater, E., M.A. Soto, G.A. Salazar, R. Jiménez, M.A. López & R.L. Dressler. 2005. Las orquídeas de México. Instituto Chinoín, México, D. F. 302 p.

Martínez, P.A. 1985. Inducción *in vitro* de brotación múltiple en *Bletia urbana* Dressler (Orchidaceae) a partir de protocormos seccionados. Tesis Profesional. Facultad de Ciencias, UNAM. México. 66 p.

Martínez, P.A. 1991. Propagación masiva *in vitro* y recuperación de poblaciones de orquídeas en peligro de extinción. Tesis de Maestria. Facultad de Ciencias, UNAM. México. 107p.

Ortega-Larrocea, M.P., S.V.M. Luna-Rosales & V.M. Chávez-Avila. 2000. Potencialidad de la micorrización masiva *in vitro* en la micropropagación de *Bletia urbana* (Orchidaceae). *In:* Resúmenes de la 1era. Reunión Iberoamericana y III Simposio Nacional sobre Micorriza, Guanajuato, México. Pp. 28.

Ortega-Larrocea M. P. 2004. Symbiotic reintroduction of terrestrial orchids in Mexico. II International Orchid Conservation Congress, Sarasota, Florida.

Ramsay, M.M. & K.W. Dixon. 2003. Propagation science, recovery and translocation of terrestrial orchids. Pp. 259-286 *in*: K.W. Dixon, S.P. Kell, R.L. Barret & P.J. Cribb. (eds.). Orchid conservation. Natural History Publications, (Borneo), Kota Kinabalu, Sabah.

Ramsay, M.M. & J. Stewart. 1998. Re-establishment of the lady´s slipper orchid (*Cypripedium calceolus* L.) in Britain. Bot. J. Linn. Soc. 126: 173-181.

Rangel, V.M. 2004. Aislamiento de hongos micorrízicos de orquídeas terrestres de la Reserva "El Pedregal" de San Angel, México. D. F. Tesis de Licenciatura, Facultad de Estudios Superiores, Iztacala, Universidad Nacional Autónoma de México. 113 p.

Rangel, V.M. 2006. Germinación simbiótica y reintroducción de orquídeas terrestres en la Reserva ecológica del Pedregal de San Angel, México, México D. F. Tesis de

maestría, Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México. 115 p.

Rubluo, A., V. Chávez & A. Martínez. 1989. *In vitro* seed germination and re-introduction of *Bletia urbana* (Orchidaceae) in its natural habitat. Lindleyana 4: 68-73.

Rubluo, A., V. Chávez, A.P. Martínez & O. Martínez-Vázquez. 1993. Strategies for the recovery of endangered orchids and cacti through *in vitro* culture. Biol. Conserv. 63: 163-169.

Schuiteman, A. & E.Vogel. 2003. Pp. 55-68 *in*: K.W. Dixon, S.P. Kell, R.L. Barret & P.J. Cribb. (eds.). Orchid conservation. Natural History Publications, (Borneo), Kota Kinabalu, Sabah.

Stewart, S.L., L.W. Zettler & J. Minso. 2003. Symbiotic germination and reintroduction of *Spiranthes brevilabris* Lindley, an endangered orchid native to Florida. Selbyana 24: 64-70.

Téllez-Velasco, A. 2002. The Pedregal of San Angel and its orchids. Orch. Rev. 110: 25-9.

Whigham, D.F., J.P. O´Neill, H.N. Rasmussen, B.A. Caldwell & M.K. McCormick. 2006. Seed longevity in terrestrial orchids – potential for persistent *in situ* seed banks. Biol. Conserv. 129: 24-30.

Zettler, L.W., J. Sharma, F.N. Rasmussen. 2003. Mycorrhizal diversity. Pp. 205-226 *in*: K.W. Dixon, S.P. Kell, R.L. Barret & P.J. Cribb. (eds.). Orchid conservation. Natural History Publications, (Borneo), Kota Kinabalu, Sabah.

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ESTUDIO DE BACTERIAS ASOCIADAS A ORQUÍDEAS (ORCHIDACEAE)

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México cuenta con 1 106 especies y subespecies de orquídeas distribuidas en 159 géneros, de las cuales 444 especies y subespecies son endémicas representando el 40% de los taxas registrados en el país. Esta diversidad constituye un enorme potencial ornamental, que hasta la fecha no se ha aprovechado en toda su magnitud por los mexicanos, especialmente por sectores de la sociedad que cuentan con estos recursos fitogenéticos (Red de ornamentales 2004, Soto 1996)

Las orquídeas representan la cúspide de procesos evolutivos y ecológicos dentro del reino de las plantas, ya que han desarrollado un amplio potencial evolutivo para su adaptación que si bien les ha permitido aprovechar un recurso y ocupar ciertos nichos, también las hace ser muy vulnerables ante los cambios en su ambiente, de la cual son objeto a través de la colecta indiscriminada; destrucción, modificación y fragmentación de su hábitat, estas características provocan bajas tasas de crecimiento, ciclos de vida relativamente largos y escaso reclutamiento de nuevos individuos en condiciones naturales así como el establecimiento de asociaciones con polinizadores, micorrizas y con otros organismos que son a veces tan específicas y complejas. (IUCN/SSC 1996; Ospina 1996).

Dentro de estas asociaciones poco conocidas, pero no por eso menos importantes son las que han establecido las orquídeas con bacterias, estas se han encontrado dentro del sustrato donde se desarrollan y en las raíces de orquídeas tanto epífitas como terrestres. De los géneros bacterianos aislados de estas orquídeas, se ha demostrado que *Azotobacter* y la bacteria *Bacillus radicicola* promueven la germinación de semillas de orquídeas por la producción de la fitohormona auxina (IAA). Y bacterias de los géneros *Pseudomonas*, *Bacillus, Arthrobacter*, y *Xanthomonas*) y *Sphingomonas* sp, *Microbacterium* sp., *Mycobacterium* sp., *Bacillus* sp., *Rhizobium* sp., *Rhodococcus* sp., *Cellulomonas* sp., *Pseudomonas* sp., y *Micrococcus luteus* también tienen la capacidad de producir esta hormona. (Knudson, 1922; Wilkinson *et al.* 1989 y 1994; Tsavkelova *et al*. 2004a, 2004 b).

Por otra parte se tiene conocimiento del empleo de biofertilizantes en plantas de importancia agrícola como maíz, trigo, caña de azúcar, arroz, café y tomate entre otros, donde se han observado cambios importantes al aplicarse estos, en el proceso de germinación, desarrollo y producción de los cultivos, los biofertilizantes son producidos por diferentes géneros bacterianos: *Azospirillum*, *Acetobacter, Azotobacter*, *Bacillus*, *Klebsiella*, *Rhizobium, Pseudomonas y Serratia*, estas respuestas se deben a que los microorganismos aumentan el reciclado y la solubilización de los nutrientes minerales y sintetizan vitaminas, aminoácidos, auxinas, giberelinas, citoquininas y etileno; así como algunas de ellas fijan nitrógeno reduciendo de manera importante el uso de fertilizantes químicos cuyo uso a largo plazo tienen efectos negativos para el ambiente (Frankenberger & Arshad 1995, Fuentes *et al*. 2003, Jiménez Salgado *et al.* 2004).

Aunque la composición de la microbiota asociada a orquídeas es de limitada especificidad, es importante realizar estudios sobre las poblaciones bacterianas que se encuentren asociadas a orquídeas en ambientes silvestres , ya que esta varía en función de las condiciones ambientales y del nicho ecológico en que se encuentren, además de las características morfológicas y fisiológicas que presentan las plantas y permitan el establecimiento de tales asociaciones. (Wilkinson *et al*. 1994).

En este trabajo se planteó estudiar la población de bacterias que se encuentran asociadas a dos especies de orquídeas *Laelia furfuracea*, especie endémica del estado de Oaxaca que crece en bosques de pino-encino, en clima frío seco y *Oncidium sphacelatum* especie tropical de amplia distribución.

TABLA 1.Géneros bacterianos aislados de plantas de *Laelia furfuracea.*

	Rizósfera	Rizoplano	Endófito Raíz	Endófito Hoja	Endófito Pseudobulbo	Total n=67
Azospirillum	4 28.6%)	4 (28.6%)	3 (21.4%)	2 (14.3%)	1 (7.1%)	14 (20.9%)
Enterobacter	4 (22.2)	4 (22.2)	5 (27.8)	2 (11.1)	3 (16.79	18 (26.9%)
Pseudomonas	3 (25%)	3 (25%)	3 (25%)	3 (25%)	3 (25%)	12 (17.9%)
Acetobactereas	2 (16.7%)	3 (25%)	3 (25%)	1 (8.3%)	3 (25%)	12 (17.9%)
Herbaspirillum	3 (27.3%)	2 (18.2%)	3 (27.3%)	2 (18.2%)	1 (9.1%)	11 (16.4%)

TABLA 2.Géneros bacterianos aislados de plantas de *Oncidium sphacelatum.*

	Rizósfera	Rizoplano	Endófito Raíz	Endófito Hoja	Endófito Pseudobulbo	Total n=81
Azospirillum	4 (20%)	4 (20%)	4 (20%)	4 (20%)	4 (20%)	20 (24.7%)
Enterobacter	3 (21.4)	4 (28.6%)	4 (28.6)	3 (21.4%)	ND	14 (17.3%)
Pseudomonas	2 (15.4%)	4(30.8%)	3 (23.1%)	3 (23.1%)	1 (7.7%)	13 (16%)
Acetobactereas	4 (22.2%)	4 (22.2%)	4 (22.2%)	4 (22.2%)	2 (11.1%)	18 (22.2%)
Herbaspirillum	3 (18.8%)	5 (31.3%)	5 (31.3%)	3 (18.8%)	ND	16 (19.8%)

ND = No hubo aislamiento

Material y Métodos

La recolección de plantas de *Laelia furfuracea* se realizó en la parte noreste del estado Oaxaca en agosto del 2005 y de *Oncidium sphacelatum* en la sierra Nororiental del Estado de Puebla en marzo de 2006. Se recolectaron 4 plantas en cada uno de los sitios. El aislamiento de bacterias se hizo en las diferentes zonas anatómicas de las orquídeas: rizósfera, rizoplano y endófitos que corresponden a pseudobulbo (tallo), hoja y rizoma, lavados y desinfectados con una solución de Hipoclorito de Sodio al 1% / 5 minutos, se enjuagó con agua destilada estéril (4 veces) y se maceró. Se procedió a inocular los viales con 100 µl de cada dilución en los medios: Nfb, para aislar *Azospirillum*; HNfb, para aislar *Herbaspirillum*; Mconkey en caldo e Hino y Wilson, para *Klebsiella* y bacterias entéricas; P4, para *Pseudomonas*; y LGI para *Acetobacter* se incubaron a 30º C durante 24-72 horas, transcurrido el tiempo de incubación se procedió a revisar el crecimiento característico en cada uno de los medios. En esta primera etapa se determinó la población bacteriana existente en las plantas de las orquídeas. Los viales positivos fueron resembrados en los mismos medios y se tomo la lectura de estos, posteriormente se tomo una asada y se sembraron por estría cruzada en los diferentes medios selectivos para cada uno de los géneros de bacterias: *Azospirillum*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas* y *Acetobacter.*

Resultados

En las tablas 1, 2 y 3 se presentan las frecuencias y poblaciones de los géneros bacterianos aislados. En las muestras de orquídeas de *Laelia furfuracea* se encontraron 67 cepas bacterias de 5 géneros bacterianos. El género bacteriano con mayor frecuencia de aislamiento fue *Enterobacter* encontrándose en el interior de la raíz, seguido por *Azospirillum* en rizósfera y rizoplano, *Pseudomonas* en rizoplano *y Acetobacter* fue menor en rizósfera e interior de la raíz, finalmente *Herbaspirillum* se encontró en un 16.4% siendo el menos frecuente y con mayor número de aislados en la zona de la rizósfera e interior de la raíz (tabla 1). En las plantas de *Oncidium Sphacelatum se* logró aislar un total de 81 cepas de bacterias, a diferencia de *Laelia furfuracea* el grupo con mayor frecuencia fue *Azospirillum* (24.7%), seguido de las Acetobacter (22.2%) presentó una frecuencia de aislamiento constante en las diferentes zonas a excepción de pseudobulbo*;* La mayor frecuencia de aislamiento de los géneros bacterianos correspondió a *Pseudomonas* con un (30.8%) en la

TABLA 3. Población de microorganismos en log(células/ gramo de peso fresco) aislados de las diferentes plantas de orquídeas de *Laelia furfuracea* (Oaxaca) y *Oncidium Sphacelatum* (Puebla).

Zona de la planta	Lugar de muestreo	*Azospirillum*	*Enterobacter*	*Pseudomonas*	*Acetobactereas*	*Herbaspirillum*
Rizósfera	Oaxaca	4.18	7.04	6.3	6.15	7.15
	Puebla	6.04	6.04	5.88	5.15	5.15
Rizoplano	Oaxaca	7.15	7.15	6.3	6.15	7.15
	Puebla	7.15	7.15	7.15	7.15	7.15
Endófito Raíz	Oaxaca	5.15	7.15	6.15	6.15	5.18
	Puebla	7.15	7.15	7.15	7.15	7.15
Endófito Hoja	Oaxaca	2.65	2.65	4.04	4.04	4.65
	Puebla	5.7	5.18	5.06	4.78	4.18
Endófito Pseudobulbo	Oaxaca	ND	ND	2.65	2.65	3.18
	Puebla	ND	ND	2.65	2.65	3.18

ND = No hubo aislamiento

zona del rizoplano, se puede observar en la tabla 2 que la frecuencia en la zona de pseudobulbo fue baja y nula para algunos microorganismos como *Enterobacter* y *Herbaspirillum.*

En relación a las poblaciones (tabla 3) en *Laelia furfuracea,* las mayores poblaciones se encontraron en rizósfera y rizoplano, en cambio en *Oncidium sphacelatum* se encontraron en rizoplano e interior de la raíz. En *Laelia furfuracea* el género bacteriano con mayor población en rizósfera fue *Herbaspirillum* (7.15 log cel/gr de peso fresco) y en *Oncidium sphacelatum* fueron *Azospirillum* y *Acetobacter* (6.04 log cel/gr de peso fresco). En *Oncidium sp.* en rizoplano, las poblaciones fueron constantes y elevadas para los 5 géneros (7.15 log cel/gr de peso fresco) y en Laelia sp., *Acetobacter* y *Pseudomonas* tuvieron una menor población (6.15 y 6.3 log cel/gr de peso fresco respectivamente).

En cuanto a microorganismos endófitos, se encontró que *Enterobacter* tuvo una mayor población en *Laelia sp.* en el interior de la raíz (7.15 log cel/gr) y en *Oncidium sp.* los 5 géneros presentaron las mismas poblaciones (7.15 log cel/gr). Al interior de la hoja las poblaciones disminuyeron en las dos especies de orquídeas, sin embargo en *Laelia sp.* el género más representativo fue *Herbaspirillum* (4.65 log cel/gr), y para *Oncidium sp.* fue *Azospirillum* (5.7 log cel/gr). En el interior de pseudobulbo las poblaciones de las bacterias disminuyen y no se detectan ni *Azospirillum* ni *Enterobacter*; *Acetobacter* y *Pseudomonas* presentan una población de 2.65 log cel/gr y *Herbaspirillum* presenta la mayor población dentro de los géneros (3.18 log cel/gr), para ambas especies de orquídeas (tabla 3).

Los datos anteriores son de los primeros informes sobre la asociación de Enterobacterias, *Pseudomonas* y microorganismos fijadores de nitrógeno (*Azospirillum, Enterobacter* y *Herbaspirillum*) en este tipo de cultivos como los que se tienen sobre la asociación de cultivos de tipo perenne como el café con *Acetobacter diazotrophicus* (Jiménez y col.,19979 y *Azospirillum (*Jiménez,Salgado y col 2004)..En contraste existen numerosos reportes sobre la asociación benéfica de enterobacterias, azospirilla y pseudomonas con cultivos económicamente importantes y de ciclo corto como maíz, trigo, caña de azúcar y arroz entre otros (Okon y Labandera-González, 1994; Haahtela y col, 1988; Barraquio, 1983).

La rizósfera ha sido el lugar donde se han encontrado la mayoría de los microorganismos que han resultado benéficos para los cultivos (Sundaram y col., 1988), aunque también es ahí donde habitan bacterias que pueden ocasionar daño a la planta hospedera. Los microorganismos se encuentran localizados sobre la capa mucilaginosa de la superficie de la raíz y en los tejidos superficiales de la misma, de donde reciben carbono orgánico en forma de exudados, secreciones y tejidos muertos. La presencia de bacterias en las otras zonas anatómicas de las orquídeas indica que existe un mecanismo por el cual pueden llegar al interior, así como su capacidad para poder adaptarse a las nuevas condiciones imperantes a su alrededor. La situación actual de la bacteria y su abundancia dentro

de la raíz no se conoce perfectamente pero su capacidad de sobrevivir en el interior de las plantas con poca o ninguna competencia las hace candidatos potenciales para el control biológico (Misagui & Donndelinger 1990).

LITERATURA CITADA

Barraquio W.L., J.K. Ladha & I. Watanabe. 1983. Isolation and identification of N_2-fixing *Pseudomonas* associated with wetland rice. Can. J. Microbiol. 29 : 867-873.

Frankenberger W.T. & M. Arshad. 1995. Phytohormones in soils; microbial production and Function. Marcel Dekker, New York

Fuentes R.L.E., H.A. Tapia, S.T. Jiménez, E.M.A. Mascarúa, P.Y. Santoyo, V.L.R. Caso, H.H.T. Romero, E.M. del R. Cajica, B.D. León, P.M. Rosales, M.P. Füguemann & R.M.G. Castillo. 2003. Bacterias acéticas: diversidad e interacción con las plantas, Elementos 49(10): 47.

Haahtela K., T. Laakso, E.L. Nurmiaho & T. Korhonen. 1988. Effects of inoculation of *Poa pratensis* and *Triticum aestivum* with root-associated, N2-fixing *Klebsiella, Enterobacter* and *Azospirillum*. Plant and Soil. 106: 239-247.

IUCN/SSC Orchid Specialist Group. 1996. Orchids – Status survey and conservation action plan. IUCN, Gland Switzerland and Cambridge , UK.

Jiménez S.L., C.H. Vázquez & H.A. Tapia. 2001. Response of the coffee plant to the inoculation with *Azospirillum* sp. *In*: Nitrogen fixation: global perpectives. Proceedings of the 13th International Congress on nitrogen fixation.Hamilton, Ontario, Canada.

Jiménez Salgado T., L.E. Fuentes-Ramírez, L.E., A. Tapia-Hernández, E.M.A. Mascarúa-Esparza, E. Martínez-Romero & J. Caballero Mellado. 1997. *Coffea arabica* L., new host plant for *Acetobacter diazotrophicus* and isolation of other nitrogen fixing acetobacteria. Appl. Environ. Microbiol. 63(9): 3676-3683.

Knudson, L., 1922. Nonsymbiotic Germination of Orchid Seeds, *Bot. Gaz. 73*(1): 1–25.

Misaghi I.J. & C.R. Donndelinger. 1990. Endophytic bacteria in symptom-free cotton plants. Phytopathology 80: 808-811.

Okon Y. & C.R. Labandera-González. 1994. Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. Soil. Biol. Biochem. 26: 1591-1601.

Ospina, H. M., 1996, Orchidology and Biotechnology, Orchids: 1072-1074.

Red de ornamentales 2004. (http://www.uaemex.mx/ornamentalesred/home.html)

Soto-Arenas, M. A. 1996. México (Regional account). Pp. 53-58 *in*: IUCN/SSC Orchid Specialist Group. Orchids. Status survey and conservation action plan, UICN.

Sundaram S., A. Araunakumari & R. Klucas. 1988. Characterization of Azospirilla isolated from seeds and roots of turf grass. Can. J. Microbiol. 34 : 212-217.

Tsavkelova, E.A., T.A. Cherdyntseva & A.I. Netrusov. 2004a. Bacteria associated with roots of epiphytic orchids. Mikrobiologiya 73(6): 825–831.

Tsavkelova, E.A., T.A. Cherdyntseva & A.I. Netrusov. 2004b. Auxin production by bacteria associated with orchid roots. Microbiology 74(1): 46–53, translated from Mikrobiologiya 74(1): 55–62.

Wilkinson K. H., K.W. Dixon & K. Sivasithamparam. 1989. Interaction of soil bacteria, mycorrhizal fungi and orchid seed in relation to germination of Australian orchids. New Phytol., 112: 429-435

Wilkinson, K.G., K.W. Dixon, K. Sivasithamparam & E.L. Ghisalberti. 1994. Effect of IAA on symbiotic germination of an Australian orchid and its production by orchid-associated bacteria. Plant Soil 159: 291–295.

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EFFORTS TO CONSERVE ENDANGERED TERRESTRIAL ORCHIDS *IN SITU* AND *EX SITU* AT TWO NATURAL RESERVES WITHIN CENTRAL MEXICO

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The natural vegetation in and around Mexico City once harbored an unusually high number of plant and animal (insect) species, including endemics (Vázquez 1973, Ceballos & Galindo 1984, Rzedowski 1991). The high diversity in this region has been attributed to the unusual topography resulting from a series of volcanic eruptions that ended *ca.* 1800 years ago (Siebe *et al.* 2004). In addition, two phyto-geographic regions overlap within Central Mexico that support diverse vegetation types (*e.g.*, shrubs, mature pine forests). Due to the rapid, uncontrolled growth of Mexico City's population, and surrounded Cities as Cuernavaca, many of these habitats have been destroyed, prompting the establishment of several natural reserves, especially south of the city. Two reserves are the subject of this study: El Pedregal in Mexico City, and El Corredor Biológico Ajusco-Chichinautzin limited by the southern Mexico City and Northern Morelos State. El Pedregal is a relictual area (237 ha) where some representative elements of the original fauna and flora of this Valley still prevail (Valiente-Banuet & De Luna-García 1994, Téllez 2002, Castillo-Argüero *et al*. 2004, Hágsater *et al*. 2005). A xerophytic shrub vegetation is supported by a basaltic shield where any developed soil can be found other than organic matter accumulations in depressions and fissures (Cano-Santana & Meave 1996). High plant diversity was described initially by Rzedowsky (1954) (*c.a.* 350 species) and Asteraceae, Poaceae, Leguminoseae and Orchidaceae Families are the dominant (Herrera & Almeida 1994). Terrestrial orchid diversity has been documented, especially in El Pedregal and a total of 25 orchid species have been reported, including five species on the verge of extinction (*Bletia punctata, Cyrtopodium macrobulon*, *Epidendrum anisatu*, *Habenaria strictissima*, *Liparis greenwoodiana*) (Hágsater *et al.* 2005).

In contrast, in the Chichinautzin Area, eight types of vegetation can be found. An altitudinal gradient joint with successive periods of volcanic activity are combined and pedogenetic processes through time and parental material result in a chronosequence of soils. Main vegetation type is *Pinus* forest developed in elevations from 1800 to 3500 m and in an extension of 65, 700 ha. Around 785 different plant species have been described where Orchidaceae is the more diverse Family with 125 species (six are protected and 25 are listed in the IUCN-CITES and the Red List of Threatened Plants (Espejo *et al.* 2000).

The main ecological problem in the first place is the habitat fragmentation where the degradation processes are the spread of non-controlled fires during the dry season, over collection and pollution problems, such as trash dumps replacing native for perturbed flora. Meanwhile at the second place, the main degradation processes are the conversion of forest into agricultural lands as a result of overpopulation, with subsequent irrational exploitation of wood and also uncontrolled fires. In both habitats, the Orchidaceae is one of the most endangered families because of the changes in vegetation, soil use and over collection that do not allow populations to recover (Rubluo *et al*. 1993, Mera *et al*. 2002, Téllez 2002, Koopowitz *et al*. 2003, Wotavová *et al*. 2004). This problem gets worse from the fact that no governmental effort is made to preserve biodiversity in seed collections as has been done for some forest species. In consequence, there is not any future perspective to consider soil microorgan-

isms as mycorrhizal fungi in conservation strategies like habitat restoration, for this particular group (Zettler 1997). On the other hand, it is well supported and established the use of ectomycorrhizal fungi in reforestation of gymnosperm forest with macromycetes. The main problem is that local inoculation programs do not use native fungi and when done, they do it with commercial isolates.

We have conducted an extensive project aimed at monitoring, conserving germoplasma, and isolating mycorrhizal fungi from orchids at both sites. Our aim is not to begin an uncontrolled seed collection practice without the isolation of associated mycorrhizal fungi in order to promote symbiotic germination with natural isolates. Studies at El Pedregal initiated in 2002 during the rainy season and we were able to found half of the original described orquiflora for this habitat. We started a germless storage with a total of 105 capsules and 73 different collect numbers with 38 identified isolates at the anamorphic stage (Currah *et al.* 1997). Some examples are shown in Table 1, Fig. 1.

At the second place, El Corredor Chichinautzin, because of its big extension, we started to locate well conserved forest sites with contrasting soil quality. Recently (2005) we identified a total of 25 species in sites with non-developed soils similar to the El Pedregal habitat and sites with deep and well developed Andosols. In one year of field monitoring during the rainy season, we got 250 capsules (65 collected numbers) and 18 mycorrhizal isolates, all of them nearly identified in the anamorph stage (Table 1, Figs. 1 and 2). One of this unidentified isolates belonged to an achlorphylic orchid *Corallorhiza maculata.*

A variety of morphological features were found in the different isolates as well as specificity for the plant species with their mycorrhizal fungi. There is less morphological variation between the isolates found in El Pedregal probably due to the fact that they come from fewer species of orchids in a smaller habitat. A wide morphological variation was detected in the isolates from the Chichinautzin, also because they come from more orchid species and habitats. Main morphological variations are related to the rate of development and the texture of the mycelium forming the colony in the Petri dish. *Epulorhiza* spp. isolates have consistently waxy mycelium with submerged hyphae in yellowish- pale brown colors where monilioid cells are quite similar. Molecular studies have been conducted for the isolates from El Pedregal and all teleomorphic species of *Epulorhiza* belong to Tulsnellaceae, particularly *Tullasnella calospora* (Rangel 2006). For *Ceratorhiza* spp., a less specific determination was obtained to the Family Ceratobasidae. Morphological features of *Epulorhiza* spp. isolates from Corredor Chichinautzin are more cottony-texture with aerial mycelium, white and sometimes similar to *Ceratorhiza* colonies (Fig. 2B, O and R). Instead, few *Ceratorhiza* cultures grew as *Epulorrhiza,* without concentric rings and waxy texture (Fig. 1Y). *Ceratorhiza* cultures grew faster but some moniliod cells developed more slowly in some cultures and it was difficult to appreciate them because they don not finish in clumps as *Epulorhiza* (Fig. 1E1, Fig. 2H).

Although this is a preliminary study for the mycorrhizal fungi diversity associated to terrestrial orchids in southern Mexico City, we have a very clear picture of the morphological diversity that can be found associated to plant species. It looks like some genera are highly specific for their mycobiont as the couples *Bletia - Epulorhiza, Dichromanthus – Ceratorhiza, Habenaria – Epulorhiza,* and *Malaxis – Epulorhiza*. More evidence is required to confirm whether this specificity always occurs in nature, due to the fact that some symbiotic cultures can be developed *in vitro* with different isolates (Rangel 2004). Bioassays confirm specificity at some level; we have noticed that *Bletia* species are less specific for both genus isolates than *Dichromanthus.* However, results for the *in vitro* propagation must be interpreted carefully and do not reflect the specificity in nature. *In situ* bioassays demonstrate that specificity can be developed through the life history of the plant (Rangel 2006). The *Bletia* spp. can be probably less dependent on fungi for *in vitro* germination and less specific because they photosynthesize rapidly; but in nature, populations are more endangered than *Dichromantthus* and seedlings are very difficult to observe where asexual corm propagation is common (Ortega-Larrocea and Rangel, same volume). The *Ceratorhiza* fungi grew faster *in vitro*, but we do not know whether the same behavior found in

TABLE 1. Mycorrhizal isolates from several orchids in Central Mexico.

Orchid species	Number of isolates	Source plant	Site	Anamorphic phase	Growth of colony (PDA	Ranges of growth (cm/day), and l x w of moniliod cells (µm)	Figure
Bletia campanulata Lex.	2	Adult	El Pedregal	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae; waxy colony and sometimes cottony in the center	0.43 - 0.61, 12.25 x 9.8 - 14.7 x 14.7	1B-G
Bletia urbana Dressler	8	Adult	El Pedregal	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy and sometimes aerial hyphae in the margin	0.47 - 0.57	1I-M
Bletia sp. 1	1	Adult	El Pedregal	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy and sometimes cottony in the center	0.57	1N-P
Bletia sp. 2	1	Adult	Corredor Chichinautzin	*Rhizoctonia* sp.	Cream-colored to yellowish with submerged hyphae, waxy and sometimes cottony in the center	0.27	1Q-S
Dichromanthus aurantiacus (La llave & Lexarza) Salazar & Soto Arenas	23	9 from adult, 14 from protocorm	El Pedregal	*Ceratorhiza* sp.	White to cream colored, fluffy with aerial hyphae in concentric rings and sometimes in speck. Isolates obtained from adult plants and protocorms grown in both patterns	0.71 - 1.21, 24.5 x 9.8 - 31.85 x 12.25	1T-X
D. aurantiacus	2	Adult	Corredor Chichinautzin	*Ceratorhiza* sp.	Brownish to cream colored, waxy	0.56 - 0.60	1Y-Z
Dicrhomanthus cinnabarinus (Lex.) Garay	2	Adult	El Pedregal	*Ceratorhiza* sp.	Brownish to cream colored, fluffy with aerial hyphae in concentric rings	1.21	1A1-E1
D. cinnabarinus	1	Adult	Corredor Chichinautzin	*Ceratorhiza* sp.	Brownish to cream colored, fluffy with aerial hyphae in concentric rings and sometimes in speck.	0.50 - 2.12	1F1-G1
D. cinnabarinus subsp. *galeotiana*	1	Adult	Gypsum mine near Tonalá, Jalisco	n. d.	Brownish, cream-colored to yellowish with submerged hyphae, waxy	n.d.	
Galeottiella sarcoglossa (A. Rich & Galeotti) Schltr.	1	Adult	Corredor Chichinautzin	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy	0.2, 12.25 x 12.25 - 9.8 x 9.8	2 A-C
G. sarcoglossa	1	Adult	Corredor Chichinautzin	*Rhizoctonia* sp.	Cream-colored to yellowish, colony develops in form of flower with waxy zones with submerged hyphae, and aerial hyphae in the petal	1.21	2D-E

TABLE 1. Mycorrhizal isolates from several orchids in Central Mexico.

Orchid species	Number of isolates	Source plant	Site	Anamorphic phase	Growth of colony (PDA (cm/day), and l x w of moniliod cells (µm)	Ranges of growth	Figure
Govenia liliacea (Lex.) Lind.	1	Adult	Corredor Chichinautzin	*Ceratorhiza* sp.	Cream colored, fluffy with aerial hyphae	0.2	2F-H
Habenaria novemfida Lind.	1	Adult	Corredor Chichinautzin	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy and sometimes aerial hyphae in the margin	2.12, 9.8 x 9.8 - 14.7 x 12.25	2I-K
Habenaria sp.	1	Adult	Veracruz	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy	0.33	2L-M
Malaxis sp. 1	2	Adult	Corredor Chichinautzin	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy and fine aerial hyphae	0.50 - 0.53	2N-P
Malaxis sp. 3	1	Adult	Corredor Chichinautzin	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy and fine aerial hyphae	0.2	2Q-S
Schiedeella eriophora (B.L.Rob & Greenm.) Schltr.	1	Adult	Corredor Chichinautzin	*Ceratorhiza* sp.	Cream colored, fluffy with aerial hyphae	1.21, 25.5 x 9.8 - 31.85 x 12.25	2T-X
Platanthera volcanica Lind.	2	Adult	Corredor Chichinautzin	*Ceratorhiza* sp.	Cream colored, fluffy with aerial hyphae in concentric rings	1.06, 24.5 x 12.25 - 36.75 x 12.25	2Y-A1
Spiranthes sp. 1	1	Adult	Corredor Chichinautzin	*Rhizoctonia* sp.	Cream-colored to yellowish with submerged hyphae, waxy and fine aerial hyphea	1.21	2B1-D1
Corallorhiza maculata (Raf.) Raf.	1	Adult	Corredor Chichinautzin	n.d.	n.d.	n.d.	2E1-F1

n.d. = not determined

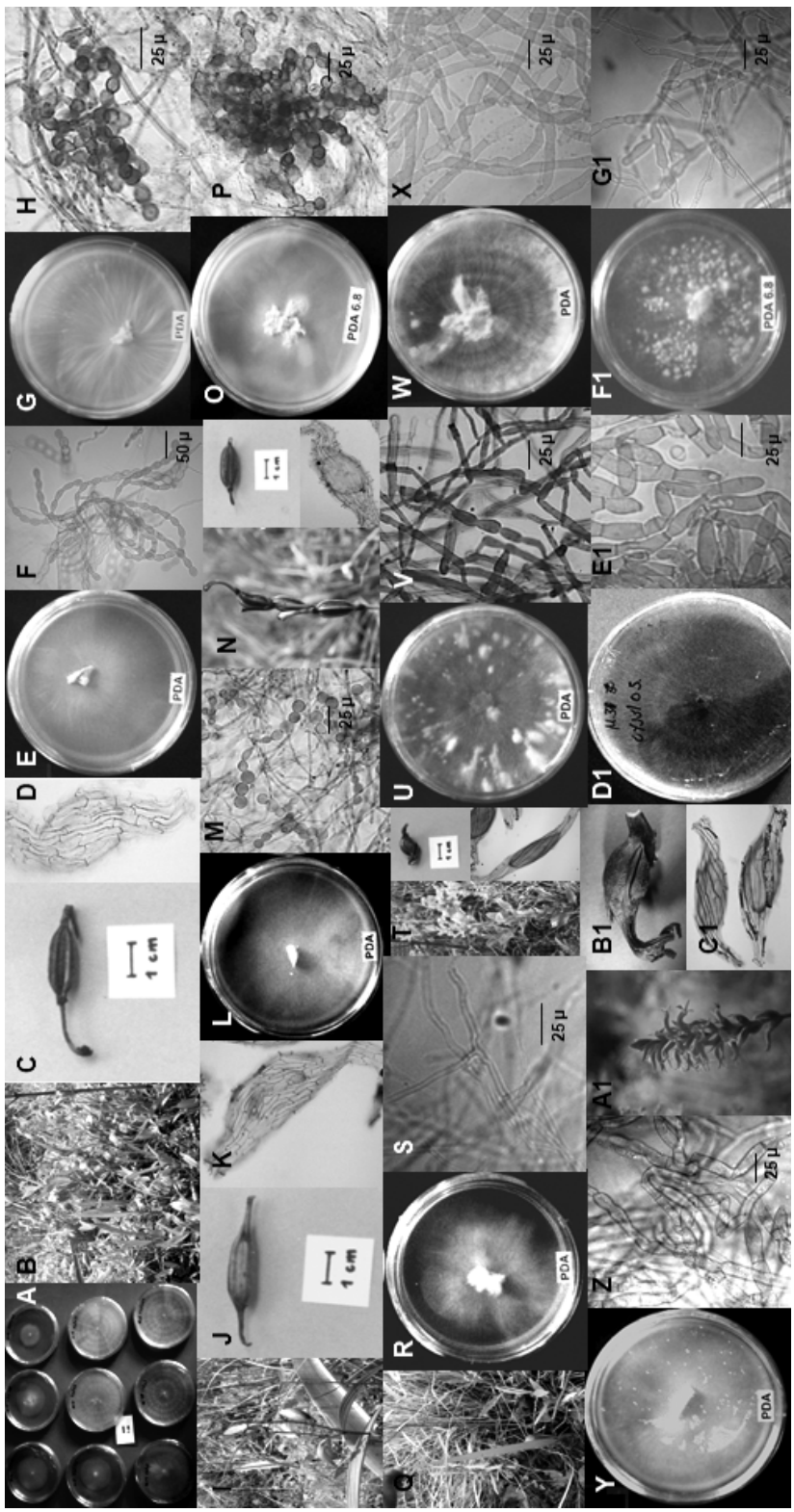

Fig. 1. **A.** General view of grow rate and colony development of mycorrhizal isolates: *Epulorhiza* spp. (first four), *Ceratorhiza* spp. (rest five). **B.** *Bletia campanulata*. **C.** Capsule. **D**. Light microscopy of seed (100 x). **E**, **G.** Colonial morphology. **F**, **H.** Microscopic morphology of monilioid cells. **I.** *Bletia urbana*. **J.** Capsule. **K.** Light microscopy of seed (100 x). **L.** Colonial morphology. **M.** Microscopic morphology of monilioid cells. **N.** Capsule of *Bletia* sp. (left), capsule detail (upper right) and light microscopy of seed (100 x) (lower right). **O.** Colonial morphology. **P.** Microscopic morphology of monilioid cells. **Q.** *Bletia* sp. **R.** Colonial morphology. **S.** Microscopic morphology of hyphae. **T.** *Dichromanthus aurantiacus* (left), capsule (upper right) and light microscopy of seed (100 x) (lower right). **U, W, Y.** Colonial morphology. **V, X, Z.** Microscopic morphology of monilioid cells. **A1.** *Dichromanthus cinnabarinus*. **B1**. Capsule. **C1.** Light microscopy of seed (100 x). **D1, F1.** Colonial morphology. **E1, G1**. Microscopic morphology of monilioid cells. Illustrations by Rangel.

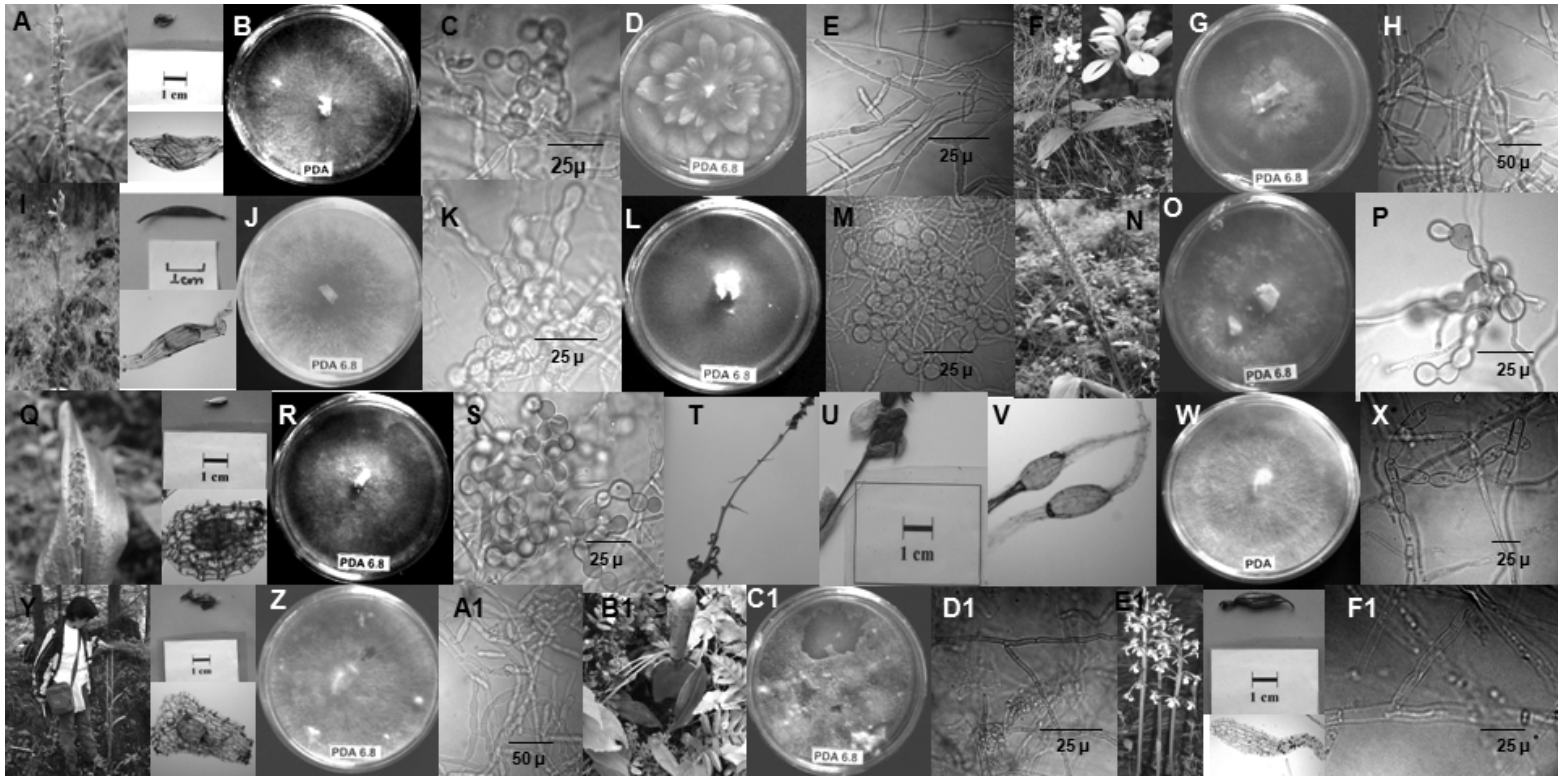

Fig. 2. **A.** *Galeottiella sarcoglossa* (left), capsule (upper right) and light microscopy seed (100 x) (lower right). **B**, **D.** Colonial morphology. **C**, **E.** Microscopic morphology of monillioid cells and mycelium. **F.** *Govenia liliacea.* **G.** Colonial morphology. **H.** Microscopic morphology of monillioid cells. **I**. *Habenaria novemfida* (left), capsule (upper right) and light microscopy of seed (100 x) (lower right). **J, L** Colonial morphology. **K, M.** Microscopic morphology of monilioid cells. **N.** *Malaxis* sp. 1, **O.** Colonial morphology. **P.** Microscopic morphology of monilioid cells. **Q.** *Malaxis* sp. 3 (left), capsule (upper right) and light microscopy of seed (100 x) (lower right). **R.** Colonial morphology. **S.** Microscopic morphology of monilioid cells. **T**. *Schiedeella eriophora* (herbarium) **U.** Herbarium flower. **V.** Light microscopy of seeds. **W.** Colony morphology. **X**. Microscopic morphology of monilioid cells. **Y**. *Platanthera volcanica* (right), capsule (upper right) and light microscopy of seed (100 x). **Z.** Colony morphology. **A1.** Microscopic morphology of monilioid cells. **B1**. *Spiranthes* sp. 1. **C1.** Coloby morphology. **D1.** Microscopic morphology of mycelium. **E1.** *Coralorrhiza maculata* (left), capsule (upper right) and light microscopy of seed (100 x). **F1.** Microscopic morphology of hyphae. Illustrations by Rangel.

soil conditions and if soil seed storage has high probability to find compatible fungus partner in natural conditions.

Additional to the studies of symbiotic and asymbiotic germination *in vitro* and *in situ*, we have also conducted studies of reintroduction, in the Ecological Reserve el Pedregal (see Ortega-Larrocea and Rangel in the same volume), showing the relevance of mycorrhizal fungi in the development and survival of orchids. This is the first Mexican report that uses a combination of strategies (*e.g*., germplasm preservation, fungal isolation, database recording) to promote orchid conservation both *in situ* and *ex situ*. The main aim of this project is to initiate a global conservation program of symbiotic fungi diversity where a collection of seeds is necessary to test the symbiotic effectiveness of the isolates. With the reintroduction results, we intend to attire the attention of the main organisms promoting orchid conservation in Mexico and present convincing evidence that mycorrhizal fungi are necessary for any realistic conservation project. We will conduct future research to demonstrate that habitat degradation decreases the biodiversity functions of soil and fungi potential and in consequence, the ability of orchids to survive in nature. We are conscious that this will be a long-term project and at the present, we attempt only easy *in vitro* bioassays with the recently collected material testing seed and fungi viability, isolate effectiveness and isolate diversity and specificity.

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LITERATURE CITED

Cano-Santana, Z. & J.C. Meave. 1996. Sucesión primaria en derrames volcánicos: el caso del Xitle. Ciencias 41: 58-68.

Castillo-Argüero, S., G. Montes-Cartas, M.A. Romero-Romero, Y. Martínez-Orea, P. Guadarrama-Chávez, I. Sánchez-Gallén & O. Núñez-Castillo. 2004. Dinámica y conservación de la flora del matorral Xerófilo de la reserva del Pedregal de San Ángel (DF, México). Bol. Soc. Bot. México 74: 51-75.

Ceballos, G. & C. Galindo. 1984. Mamíferos silvestres de la Cuenca de México. Ed. Limusa-MAB, México. 299 p.

Currah, R.S., C.D. Zelmer, S. Hambleton & K.A. Richardson. 1997. Fungi from orchid mycorrhizas Pp. 117-170 *in*: J. Arditti A.M. Pridgeon (eds.), Orchid Biology: Reviews and Perspectives, VII. Kluwer Academic Publishers, Grat Britain.

Espejo, S.A., C.J. García, F.A. López, M.R. Jiménez & S.L. Sánchez. 2002. Orquídeas del Estado de Morelos. Orquídea (Méx.) 16: 332 p.

Hágsater, E., M.A. Soto, G.A. Salazar, R. Jiménez, M.A. López & R.L. Dressler. 2005. Las orquídeas de México. Instituto Chinoín, México, D. F. 302 p.

Herrera, A.L. & L.L. Almeida. 1994. Relaciones fitogeográficas de la flora vascular de la reserva del Pedregal de San Ángel, México DF. Pp. 83-89 *in*: A. Rojo (comp.), Reserva Ecológica "El Pedregal" de San Ángel: Ecología, Historia Natural y Manejo. UNAM, México. D.F. pp 83-90.

Koopowitz, H., P.S. Lavarack & K.W. Dixon. 2003. The nature of threats to orchid conservation. Pp. 25-42 *in*: K.W. Dixon, S.P. Kell, R.L. Barrett & P.J. Cribb. Orchid conservation. Natural History Publications. Malaysia.

Mera, L.M., M.A.A. Zarate & Y.A. Sandoval. 2002. El herbarium del pedregal de San Angel. Ciencias 67: 42-43.

Rangel, V.M. 2004. Aislamiento de hongos micorrízicos de orquídeas terrestres de la Reserva "El Pedregal" de San Angel, México. D. F. Tesis de Licenciatura, Facultad de Estudios Superiores, Iztacala, Universidad Nacional Autónoma de México. 113 p.

Rangel, V. M. 2006. Germinación simbiótica y reintroducción de orquídeas terrestres en la Reserva ecológica del Pedregal de San Angel, México, México D. F. Tesis de maestría, Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México. 115 p.

Rubluo, A., V. Chávez, A.P. Martínez & O. Martínez-Vázquez. 1993. Strategies for the recovery of endangered orchids and cacti through *in vitro* culture. Biol. Conserv. 63: 163-169.

Rzedowski, J. 1954. Vegetación del Pedregal de San Angel (D. F. México). An. Esc. Nac. Cienc. Biol. IPN, 8: 59-129. México.

Rzedowski, J. 1991. El endemismo de la flora fanerogámica mexicana: una apreciación analítica preliminar. Acta Bot. Mex.: 47-64.

Siebe, C., V. Rodríguez-Lara, P. SCAF & M. Abrams. 2004. Radiocarbon ages of Holocene Pelado, Guespalapa and Chichinautzin scoria cones, south of Mexico city: Implications for archaeology and future hazards. Bull. Volcanol. 66: 203-225.

Téllez-Velasco, M.A. 2002. The Pedregal of San Ángel and its orchids. Orch. Rev. 110: 25-29.

Valiente-Banuet A. & E. De Luna-García. 1994. Una lista florística para la reserva del Pedregal de San Ángel, México DF. Pp. 67-89 *in*: A. Rojo (comp.), Reserva Ecológica "El Pedregal" de San Ángel: Ecología, Historia Natural y Manejo. UNAM, México, D.F.

Vázquez, L. 1973. Estudios sobre algunos aspectos taxonómicos y ecológicos de insectos del Pedregal de San Angel, México D. F. Folia Entomol. 25-6: 102-104.

Wotavová, K., Z. Balounová & P. Kindlmann. 2004. Factors affecting persistence of terrestrial orchids in wet meadows and implications for their conservation in a changing agricultural landscape. Biol. Conserv. 118: 271-279.

Zettler LW. 1997. Terrestrial orchid conservation by symbiotic seed germination: Techniques and perspectives. Selbyana 18(2): 188-194.

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TROPHIC RELATIONSHIPS IN ORCHID MYCORRHIZA – DIVERSITY AND IMPLICATIONS FOR CONSERVATION

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Introduction

Orchid species are perennial, and though demographic data suggest that the family includes r- as well as K-strategists (Whigham & Willems 2003), most species are potentially long-lived. Individual plants may be kept in living plant collections or in nature reserves for practically unlimited periods of time. There are several reports on natural populations suspected of little or no seedling recruitment, "senile populations" (Tamm 1991, Rasmussen 1995), especially among rare orchids under critical surveyance. Such populations may function as a seed source to neighbouring areas but are likely to eventually disappear from the site.

Sustainable conservation thus requires the preservation of conditions that enable the species to carry through its entire life cycle. The conservational concern should also involve species of other organisms that are associated during a critical life stage, such as a pollinator during flowering, or a symbiotic fungus during seed germination (Zettler *et al.* 2003). Not only that, but the requirements of these organisms must be considered, such as appropriate substrates for the fungi. Clearly, "orchids require an ecosystem approach to their conservation" (Roberts 2003).

In light of recent research, the orchid-fungus relationship has proved particularly complex, as it may be subject to trophic changes during the lifetime of the orchid. The degree of specificity, and the paths of biological energy are major concerns in these relationships. All of this may need to be assessed in cases of severely endangered orchid species.

Orchid mycorrhiza is still considered a unilateral relationship

Transport of carbohydrates from fungi to seedlings of orchids has been amply demonstrated, beginning with Smith's experiments (1966, 1967). There is no other feasible explanation for the long-term deficiency in the photoassimilating apparatus known from orchid seedlings generally and adult stages widespread in the family (e.g, Girlanda *et al*. 2006). Recent stable isotope analyses support the fungal origin of a significant part of the C and N found in aboveground structures of orchids (Gebauer & Meyer 2003*,* Julou *et al.* 2005). Hyphal coils within the orchid tissues become degraded by an enzymatic process and transfer is assumed to occur entirely or predominantly over a dead fungal interface, as ultrastructural studies suggest (Peterson *et al.* 1996). This further adds to the evidence of an asymmetric relationship, with the orchid as the receiving and dependent part.

A recent report stating mutualism in orchid-fungus relationships (Cameron *et al.* 2006) was based on a set of special experimental circumstances: Surface sterilized plantlets were planted on an inert agar, and internal hyphae from within the rhizome were allowed to colonize the agar. When $^{14}CO_2$ was subsequently supplied to the leaves, about 2% of the photoassimilated labeled carbon could later be traced to the mycelium. Physiologically interesting as this may be, it is important to note that the result was obtained under an extreme starvation of the mycelium. Such conditions would hardly ever occur under field conditions where complex carbon

sources abound. Furthermore, we do not know whether the transfer occurred via an intact plant interface surrounding hyphal pelotons. Field studies suggest that low substrate carbon supply may increase the virulence of the fungi and turn the situation into parasitism of the fungus on the orchid (Beyrle *et al.* 1995).

Orchids are never "fully autotrophic"

Seedlings of *Neuwiedia veratrifolia*, belonging to the subfamily Apostasioideae, usually considered the most basal in orchid phylogeny, were found for the first time by Kristiansen *et al.* (2001). They develop typical protocorms with pelotons, and the fungi associated with them in the wild proved to belong to *Tulasnella* and *Thanathephorus*, two genera that are known to develop *Rhizoctonia*-stages (Kristiansen *et al.*, 2004). VAM is the only type of mycorrhiza found in monocotyledons outside of Orchidaceae, and it seems a plausible scenario that ancestors of the orchid family developed a seedling mycotrophy, based on invasive saprotrophic rhizoctonioid mycelia in conjunction with the evolution of micro-seeds. From the beginning the whole range of this rather mixed assembly of imperfect mycelia of Basidiomycetes (i.e., *Rhizoctonia* s.l., Table 1, below) appears to have been employed.

Orchid seed evolution seems to have run towards further reduction in size, the epiphytic orchid groups tending to produce smaller seeds than terrestrials (Rasmussen, 1995). Assuming an evolutionary reduction of seed nutrient reserves within the orchid family clade, a secondary loss of seedling mycotrophy appears unlikely, and is not supported by any observations so far. In other words: seedling mycotrophy seems to be a uniquely derived and omnipresent orchid character.

Plant seedlings generally begin life by utilizing seed reserves that consist of stored photoassimilates from their autotrophic mother plant. In contrast, the orchid seedling relies not only on reserves from the mother plant but also on carbohydrates from mycotrophy. Otherwise the seedlings will not develop in the field. Thus, if the whole life history is considered, orchids are never fully autotrophic. When this description is sometimes used about orchids (e.g., "the fully autotrophic *Listera ovata*", Girlanda *et al.*, 2006), this either refers to the adult stage only, or must be considered a slip of the pen.

In terms of preserving an orchid species, this means that fungi employed during germination cannot be disregarded. All orchids are to some degree mycoheterotrophic, although this designation has somewhat misleadingly been restricted to species with obviously chlorophyll-deficient adult stages. The sequential or simultaneous combination of mycotrophy and phototrophy, that is charactistic of orchids, may be described as mixotrophy. The only exception from mixotrophy would be the entirely mycotrophic orchids. *Cephalanthera damasonium* is an example of a species that segregates into holomycotrophic and mixotrophic individuals: the albinos showed no trace of photoassimilation as adult plants, whereas the adult green individuals were found to be mixotrophic with about fifty-fifty contribution of carbon from either system (Julou *et al.* 2005). Other studies, also based on the distribution of stable carbon and nitrogen isotopes, indicate that green leaved forms may aquire a significant fraction of their C and N through fungi, but that species differ considerably in this respect (Gebauer & Meyer, 2003). Thus orchids are arranged in a continuum from holomycotrophy to various degrees of mixotrophy.

Useful terminology from the animal kingdom

Taylor (2004) put it aptly: "let's be clear – we are talking about plants that consume fungi." Much confusion may arise from inadequte or misleading designations. The phytobiont (orchid) has colloquially been referred to as the 'host', notwithstanding the fact that the mycobiont is providing the meal! Even worse are anthropomorphic expressions that seem to imply voluntary and mutualistic associations ('marriage', 'fidelity', 'promiscuity'), or deception ('cheater') which suggests a previous mutualism or presupposes a "normal" behavior deviated from. Even the idea of 'specificity' implies a degree of mutual selection. Such expressions should be avoided as they are inconsistent with our observations and present knowledge.

TABLE 1. Above: Examples of orchid-fungus-substrate relationships. Below: Fungus genera mentioned above, listed with taxonomic position according to Kirk *et al.* 2001.

Orchid species	Trophic stage	Provider/Prey	Ultimate food source
Neuwiedia veratrifolia: individually monophagous? (Kristiansen *et al.* 2004)			
	seedling mycotrophy	*Tulasnella Thanathephorus*	leaf litter (Kristiansen *et al.*, 2001)
	adult mycotrophy	*Tulasnella* sp., *Thanathephorus* sp.	leaf litter
	adult phototrophy		
Cypripedium**,** several species: mono-oligophagous (Shefferton *et al.* 2005)			
	seedling mycotrophy	?	
	adult mycotrophy	Tulasnellaceae	organic debris
	adult phototrophy		
Goodyera pubescens and ***Liparis lilifolia:*** mono(-oligo)phagous (McCormick *et al.* 2004)			
	seedling mycotrophy	*Tulasnella* cf. *bifrons*	organic debris
	adult mycotrophy	*Tulasnella* cf. *bifrons*	organic debris
	adult phototrophy		
Epipactis microphylla**:** oligophagous (Selosse *et al.* 2004)			
	seedling mycotrophy	*Tuber?*	
	adult mycotrophy	mainly *Tuber* cf. e*xcavatum*	live trees (ECM)
	phototrophy (in green individuals) and not (in albinos)		
Neottia nidus-avis: oligophagous, locally monophagous? (McKendrick *et al.* 2002*,* Selosse *et al.* 2002)			
	seedling mycotrophy	*Sebacina*	live trees (ECM)
	adult mycotrophy	*Sebacina*	live trees (ECM)
	no phototrophy (sources cited in Mckendrick *et al.* 2002)		
Limodorum abortivum: oligophagous, obligate fungal switch? (Girlanda 2006)			
	seedling mycotrophy	*Ceratobasidium?*	organic debris
	adult mycotrophy	*Russula* spp.	live trees (ECM)
	very little phototrophy		
Tipularia discolor: switch from germination fungus, polyphagous as adult (McCormick *et al.* 2004)			
	seedling mycotrophy	*Tomentella* sp.	large woody debris (Rasmussen & Whigham 1998)
	adult mycotrophy	4 groups of tulasnelloids + some persistance of *Tomentella*	organic debris
	adult phototrophy		
Corallorhiza trifida: monophagous (McKendrick *et al.* 2000a+b)			
	seedling mycotrophy	*Tomentella*	*Salix* and *Betula* ECM
	adult mycotrophy	*Tomentella*	*Salix* and *Betula* ECM
	no phototrophy		
Epidendrum rigidum: monophagous (Pereira *et al.* 2005)			
	seedling mycotrophy	*Epulorhiza*	? saprophyte
	adult mycotrophy	*Epulorhiza*	? saprophyte
	adult phototrophy		
Hexalectris spicata: oligophagous (Taylor *et al.* 2003)			
	seedling mycotrophy	?	?
	adult mycotrophy	Sebacinaceae+ *Thanathephorus*	live trees (ECM)?
	chlorophyll deficient		

TABLE 1. Continue.

Orchid species	Trophic stage	Provider/Prey	Ultimate food source
Gastrodia elata: serial monophagy, obligate switch of fungus (Xu & Guo, 2000)			
	seedling mycotrophy adult mycotrophy no phototrophy	*Mycena osmundicola* *Armillaria mellea* s.l.	leaf litter live and dead wood
Epipogium roseum: oligophagy? Yamato et al. 2005			
	seedling mycotrophy adult mycotrophy no phototrophy?	? Coprinus + Psathyrella	dung, dead wood

Teleomorph	Anamorph	Family	Order and class
Armillaria mellea		Marasmiaceae	Agaricales Basidiomycetes
Ceratobasidium	*Ceratorhiza (Rhizoctonia* s.l.*)*	Ceratobasidiaceae	Ceratobasidiales Basidiomycetes
Coprinus		Coprinaceae	Agaricales Basidiomycetes
Mycena		Tricholomataceae	Agaricales Basidiomycetes
Psathyrella		Coprinaceae	Agaricales Basidiomycetes
Russula		Russulaceae	Russulales Basidiomycetes
Sebacina	*Epulorhiza (Rhizoctonia* s.l.*)*	Exidiaceae	Tremellales Basidiomycetes
Thanathephorus	*Rhizoctonia* s.str.	Ceratobasidiaceae	Ceratobasidiales Basidiomycetes
Tomentella		Thelephoraceae	Thelephorales Basidiomycetes
Tuber		Tuberaceae	Pezizales Ascomycetes
Tulasnella	*Epulorhiza (Rhizoctonia* s.l.*)*	Tulasnellaceae	Tulasnellales Basidiomycetes

It seems about time to acknowledge that orchids are mycophagous and that the orchid-fungus association is more like a predator-prey-relationship. A set of concepts and terminology from the zoological vocabulary comes to mind. Recent research has revealed a trophic diversity in orchids so great that we need these concepts to encompass the whole range. Thus, we have examples of orchids with a broad food selection (i.e. polyphagous), the diet spanning several fungal families (*Tipularia discolor,* Table 1) as well as examples of orchids that are oligophagous, utilizing a minor group of related fungi. Verification of strict monophagy requires the analysis of the plant species through much of its geographic and ecological range. Normally monophagy would be an orchid species-to-fungal species relationship but it might also exist on the individual level, as shown in *Goodyera pubescens* (McCormick *et al.* 2006). In this species germination could be carried out with a range of *Rhizoctonia* spp., but the first strain to infect an individual protocorm seemed to be subsequently preferred. Young plants of *Goodyera pubescens* only rarely switched from their initial fungus, which shows a surprising ability of seedlings to discriminate hyphae. When a switch was induced experimentally, it carried a considerable risk of mortality.

Nevertheless, an obligate switch of fungus at some point during adolescence is well documented in *Gastrodia elata,* that is known to germinate on *Mycena osmundicola* and switch to *Armillaria mellea* later (i.e. serial monophagy). There are no reports of other food sources for *G. elata* and the switch appears to be necessary for life cycle progression (Xu & Guo 2000). The same applies to *Tipularia discolor* (McCormick *et al.* 2004), along with a successional change in the growing environment (Rasmussen & Whigham 1998).

Fungal switch may be a more wide-spread phenomenon, however. The sporadic occurrence of various *Rhizoctonia* mycelia in adult orchids otherwise feeding on ectomycorrhizal fungi as noted by Taylor *et al.* (2003), Selosse *et al.* (2004) and Girlanda *et al.* (2006) might be traces of persisting germination fungi. This parallels the situation in which *Rhizoctonia* pelotons are sporadically found in orchid species that go almost entirely phototrophic soon after germination (Bayman *et al.* 2002).

Conservational implications

The identification of the fungi carries a great deal of information about the natural requirements of the orchid species, since the ultimate food sources may be identified, be it leaf litter, woody debris or certain live host trees (Table 1). In a conservation context that would enable the detection of recruitment sites or encouragement of new ones (Batty *et al.* 2001).

A broad food selection may render an orchid species comparatively robust to environmental changes. On the other hand, the generalist strategy is considered costly in terms of defence mechanisms to keep the fungi from becoming virulent. The mono- or oligophagous orchid can be optimally adapted to a narrow food selection but is more likely to experience food limitation that might prevent sexual reproduction and threaten individual survival, if photosynthesis is not a sufficient option. It would also be more dependent on the quality of this narrow food base.

Assessing the relative importance of phototrophic assimilation is also important, because this identifies the light requirements of the orchid species in question. The epilithic *Lepanthes rupestris* appears to be an example of fungal dependency ending soon after germination (Bayman *et al.* 2002), pelotons being extremely rare in the roots of young and more mature plants at two sites studied (but no leafless seedlings were seen). The same seems to apply to several species of *Cypripedium* and many epiphytic species, the canopy environment probably offering opportunities for a largely phototrophic existence. The holomycotrophic species, of course, represent the other extreme, being able to survive in deep shade or even as entirely subterraneans (*Rhizanthella*).

Over and above specific inherited trophic traits there is, of course, in many orchids a phenotypic plasticity in respect to mycotrophic persistence, which is influenced by the growing conditions offered at each site and time. For instance, a lack of mycorrhizal infection in adult plants needs not be interpreted as an inherently low dependence on mycotrophy. The plants in question could simply be optimizing their individual survival in an environment with much light and exhausted fungal food sources.

Evolutionary considerations

Rhizoctonia-based seedling mycotrophy was probably the first step in the evolution of orchid mycorrhiza, possibly from an arbuscular mycorrhiza-dependent ancestor, and hence is a plesiomorphic condition within Orchidaceae. The adult orchid would be expected to be at first predominantly phototrophic, as in non-mycorrhizal or arbuscular-dependent ancestors. However, an obvious adaptation to a rich fungal food supply and/or limiting light would be a paedomorphic extension of the

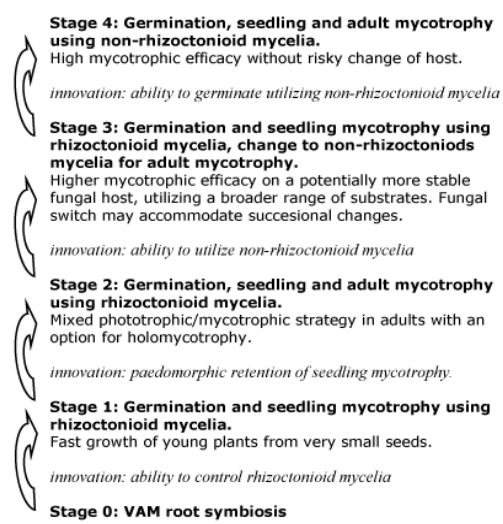

FIGURE 1. Hypothetical steps in the evolution of orchid mycorrhiza from stage 0, the non-orchid ancestor. See text for actual examples of species demonstrating the stages. Obligate ontogenetic switch of fungal host is known to occur in stage 3 orchids. It is likely that stages 1-4 have evolved several times, but it is unknown to what extent reversal may happen.

Rhizoctonia-dependency into adult life history (Fig. 1, stage 1 to 2).

Retention of this seedling mycotrophy combined with alternative fungal food sources in adult plants could be the next step in optimization of mycophagy. This evolution in orchids might be accelerated by ample available biomass of fungal species that for some reason are unable to trigger orchid seed germination. The challenges would consist of inducing initial invasion and peloton formation from mycobionts whose biology does not predispose them for entering living plant tissue, and futhermore developing novel defence mechanisms taylored to keep that infection under control (Fig. 1, stage 3).

The ultimate adaptation to such alternative food sources would be evolution of compatibility of the fungus to orchid seeds and the germination process (Fig. 1, stage 4). So far, germination by non-*Rhizoctonia* (in the broadest sense) has only been documented within a few, advanced orchid groups: *Tipularia* and *Corallorhiza, Gastrodia* (Table 1) and possibly *Cyrtosia* (*Galeola) septentrionalis* (discussed in Rasmussen, 1995).

We do not know if there is any impact on fungal fitness and evolution by this symbiosis. One might speculate that orchid predation is too slight to impact on fungal life strategies. As for the fitness, low fruiting body production has been reported in mycelia that support orchids as compared to mycelia of related fungal species (Jones & Smith, 2004, Taylor & Bruns, 1999).

Conclusions

- Orchid mycorrhiza is a non-mutualistic symbiosis and it is practical to think of it as a predator-prey or parasite-provider relationship, with the orchid as the beneficiary. Terms implying mutualism or defection from a presupposed mutualism are misleading.
- The entire life history is important in conservation of orchid species. Fungi that assist in germination are essential.
- Fungi involved in the various life phases need to be identified and their contribution to growth of the orchid assessed, be it brief or lasting, high or low.
- The ultimate food source in mycotrophy, i.e., the substrate for fungal preys needs to be rated as a maintaining factor for the orchid population in question.

LITERATURE CITED

Batty, A.L., K.W Dixon, M. Brundrett & K. Sivasithamparam. 2001. Constraints to symbiotic germination of terrestrial orchid seed in a mediterranean bushland. New Phytol. 152 : 511-520.

Bayman, P., E.J. González, J.J. Fumero & R.L. Tremblay. 2002. Are fungi necessary? How fungicides affect growth and survival of the orchid *Lepanthes rupestris* in the field. J. Ecol. 90 : 1002-1008.

Beyrle, H.F., S.E. Smith, R.L. Peterson & C.M.M. Franco. 1995. Colonization of *Orchis morio* protocorms by a mycorrhizal fungus: Effects of nitrogen nutrition and glyphosate in modifying the responses. Can. J. Bot. 73 : 1128-1140.

Cameron, D.D., J.R. Leake & D.J. Read. 2006. Mutualistic mycorrhiza in orchids: evidence from plant-fungus carbon and nitrogen transfers in the green-leaves terrestrial orchid *Goodyera repens*. New Phytol. 171 : 405-416.

Gebauer, G. & M. Meyer. 2003. ^{15}N and ^{13}C natural abundance of autotrophic and mycotrophic orchids provides insight into nitrogen and carbon gain from fungal association. New Phytol. 160 : 209-223.

Girlanda, M., M.A. Selosse, D. Cafasso, F. Brilli, S. Definer, R. Fabbian, S. Chignone, P. Pinellli, R. Segreto, F. Loreto, S. Cozzolino & S. Perotto. 2006. Inefficient photosynthesis in the Mediterranean orchid *Limodorum abortivum* is mirrored by specific association to ectomycorrhizal Russulaceae. Molec. Ecol. 15 : 491-504.

Jones, M.D. & S.E. Smith. 2004. Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms? Can. J. Bot. 82 : 1089-1109.

Julou, T., B. Burghardt, G. Gebauer, D. Berveiller, C. Damesin & M.-A. Selosse. 2005. Mixotrophy in orchids: insight from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasonium*. New Phytol. 166 : 639-653.

Kirk, P.M., P.F. Cannon, J.C. David & J.A. Stalpers. 2001. Dictionary of the fungi. 9th. ed. CAB International, Wallingford, UK.

Kristiansen, K.A., J.V. Freudenstein, F.N. Rasmussen & H.N. Rasmussen. 2004. Molecular identification of mycorrhizal fungi in *Neuwiedia veratrifolia* (Orchidaceae). Molecular Phylogenetics and Evolution 33 : 251-258.

Kristiansen, K.A., F.N. Rasmussen & H.N. Rasmussen. 2001. Seedlings of *Neuwiedia* (Orchidaceae subfamily Apostasioideae) have typical orchidaceous mycotrophic protocorms. Amer. J. Bot. 88 : 956-959

McCormick, M.K., D.F. Whigham & J. O'Neill. 2004. Mycorrhizal diversity in photosynthetic terrestrial orchids. New Phytol. 163 : 425-438.

McCormick, M.K., D.F. Whigham, D. Sloan, K. O'Malley & B. Hodkinson. 2006. Orchid-fungus fidelity: a marriage meant to last? Ecology 87 : 903-911.

McKendrick, S.L., J.R. Leake, D.L. Taylor & D.J. Read. 2000a. Symbiotic germination and development of myco-heterotrophic plants in nature: ontogeny of *Corallorhiza trifida* and characterization of its mycorrhizal fungi. New Phytol. 145 : 523-537.

McKendrick, S.L., J.R. Leake & D.J. Read. 2000b. Symbiotic germination and development of myco-heterotrophic plants in nature: transfer of carbon from ectomycorrhizal *Salix repens* and *Betula pendula* to the orchid *Corallorhiza trifida* through shared hyphal connections. New Phytol. 145 : 539-548.

McKendrick, S.L., J.R. Leake, D.L. Taylor & D.J. Read. 2002. Symbiotic germination and development of myco-heterotrophic orchid *Neottia nidus-avis* in nature and its requirement for locally distributed *Sebacina* spp. New Phytol. 154 : 233-247.

Pereira, O.L., M.C.M. Kasuya, A.C. Borges & E.F. de Araújo. 2005. Morphological and molecular characterization of mycorrhizal fungi isolated from neotropical orchids in Brazil. Can. J. Bot. 83 : 54-65.

Peterson, R.L., P. Bonfante, A. Faccio & Y. Uetake. 1996. The interface between fungal hyphae and orchid protocorm cells. Can. J. Bot. 74 : 1861-1870.

Rasmussen, H.N. 1995. Terrestrial orchids. From seed to mycotrophic plant. Cambridge University Press.

Rasmussen, H.N. & D.F. Whigham. 1998. Importance of woody debris in seed germination of *Tipularia discolor* (Orchidaceae). Amer. J. Bot. 85 : 829-834

Roberts, D.L. 2003. Pollination biology: the role of sexual reproduction in orchid conservation. Pp. 113-136. *In* Dixon, K.W., S.P. Kell, R.L. Barrett & P.J. Cribb (eds). Orchid conservation. Natural History Publications, Kota Kinabalu, Borneo.

Selosse, M.-A., M. Weiss, J.-L. Jany & A. Tillier. 2002. Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) LCM Rich. and neighbouring tree ectomycorrhizae. Molec. Ecol. 11 : 1831-1844.

Selosse, M.-A., A. Faccio, G. Scappaticci & P. Bonfante. 2004. Chlorophyllous and achlorophyllous specimens of *Epipactis microphylla* (Neottieae, Orchidaceae) are associated with extomycorrhizal septomycetes, including truffles. Microb. Ecol. 47 : 416-426.

Shefferton, R.P., M. Weiss, T. Kull & D.L. Taylor. 2005. High specificity generally characterizes mycorrhizal association in rare lady's slipper orchids, genus *Cypripedium*. Molec. Ecol. 14 : 613-626.

Smith, S.E. 1966. Physiology and ecology of orchid mycorrhizal fungi with reference to seedling nutrition. New Phytol. 65 : 488-499.

Smith, S.E. 1967. Carbohydrate translocation in orchid mycorrhizas. New Phytol. 66 : 371-378.

Tamm, C.O. 1991. Behavior of some orchid populations in a changing environment: observations on permanent plots, 1943-1990. Pp 1-13. In Wells, TCE, Willems, JH (eds), Population ecology of terrestrial orchids. The Hague: SPB Academic Publishing.

Taylor, D.L. 2004. Myco-heterotroph-fungus marriages – is fidelity over-rated? New Phytol. 163 : 217-221.

Taylor, D.L. & T.D. Bruns. 1999. Population, habitat and genetic correlates of mycorrhizal specialization in the cheating orchids *Corallorhiza maculata* and *C. mertensiana*. Molec. Ecol. 8 : 1719-1732.

Taylor, D.L., T.D. Bruns, T.M. Szaro & S.A. Hodges. 2003. Divergence in mycorrhizal specialization within *Hexalectris spicata* (Orchidaceae), a nonphotosynthetic desert orchid. Amer. J. Bot. 90 : 1168-1179

Whigham, D.F. & J.H. Willems. 2003. Demographic studies and life-history strategies of temperate terrestrial orchids as a basis for conservation. Pp. 137-158 *in*: Dixon, K.W., S.P. Kell, R.L. Barrett & P.J. Cribb (eds). Orchid conservation. Natural History Publications, Kota Kinabalu, Borneo.

Xu, J.T. & S.X. Guo. 2000. Retrospect on the research of the cultivation of *Gastrodia elata* Bl., a rare traditonal Chinese medicine. Chinese Medical Journal 113 : 686-692.

Yamato, M., T. Yagame, A. Suzuki & K. Iwase. 2005. Isolation and identification of mycorrhizal fungi associating with an achlorophyllous plant, *Epipogium roseum* (Orchidaceae). Mycoscience 46 : 73-77.

Zettler, L.W., J. Sharma & F.N. Rasmussen. 2003. Mycorrhizal diversity. Pp 205-226. In Dixon, KW, Kell, SP, Barrett, RL, Cribb, PJ (eds). Orchid conservation. Natural History Publications, Kota Kinabalu, Borneo.

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GENETIC AND MORPHOLOGICAL VARIATION IN THE *BULBOPHYLLUM EXALTATUM* (ORCHIDACEAE) COMPLEX OCCURRING IN THE BRAZILIAN "CAMPOS RUPESTRES": IMPLICATIONS FOR TAXONOMY AND BIOGEOGRAPHY

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KEY WORDS: allozymes, *Bulbophyllum exaltatum*, Cadeia do Espinhaço, campo rupestre, genetic variability, geographic barrier

Introduction

Bulbophyllum Thouars is a pantropical genus. It is one of the most species-rich genera of the Orchidaceae, with ca. 1.200 species (Dressler 1993). The genus presents myophily (pollination by Diptera) as pollination syndrome. Because orchid species are mainly self-compatible, we expect that fly-pollinated orchids present low variability within the populations and high genetic differentiation among conspecific populations, due to the reduction of the gene flow (Borba & Semir 1998, Borba *et al*. 2001). This could help to explain the high number of species in genera of fly-pollinated orchids, most of them with restricted distribution.

High montane areas of the Southeastern and Northeastern regions of Brazil, mainly in the "campos rupestres" vegetation, are the habitat for a species complex within *Bulbophyllum* sect. *Didactyle*, in which traditionally ca. 15 rupicolous species were recognized. The species of this group are vegetative uniform, being separated exclusively by the floral morphology, mainly lip differences. The main taxonomic problem in this group is the delimitation of *B. involutum*, *B. ipanemense*, *B. longispicatum*, *B. geraense* and *B. warmingianum*.

In the present study, we carried out population genetic studies using isozyme markers in 601 individuals of 33 natural populations, in order to assess the genetic variation and degree of differentiation in some species belonging to this complex. We also performed a morphometric analysis in some of the individuals of the genetic study, using multivariate methods as an attempt to improve species delimitation. Vouchers for each population were deposited in the herbarium of the Universidade Estadual de Feira de Santana (HUEFS).

Results and discussion

The four species studied, considered *a priori* to be *B. exaltatum, B. involutum*, *B. sanderianum* and *B. weddellii*, displayed high genetic variation (H_e 0.086 - 0.404) and a high degree of genetic structure (F_{ST} 0.145 - 0.269) which indicates restricted gene flow. The latter was detected only among populations of *B. exaltatum*, and is probably due to long-distance seed dispersion by wind. However, habitat fragmentation can be a factor even more important for the differentiation of these populations.

In the results of the isozyme analysis, none of the conspecific populations were formed a distinct cluster. Therefore, based on these data, a clear taxonomic delimitation among the four species considered is not possible within this complex. On the other hand, the populations clustered primarily based on the State of origin, which correspond to the two main disjunt areas of campos rupestres in Minas Gerais and Bahia (Fig. 1). The inversion of the relative frequency of the

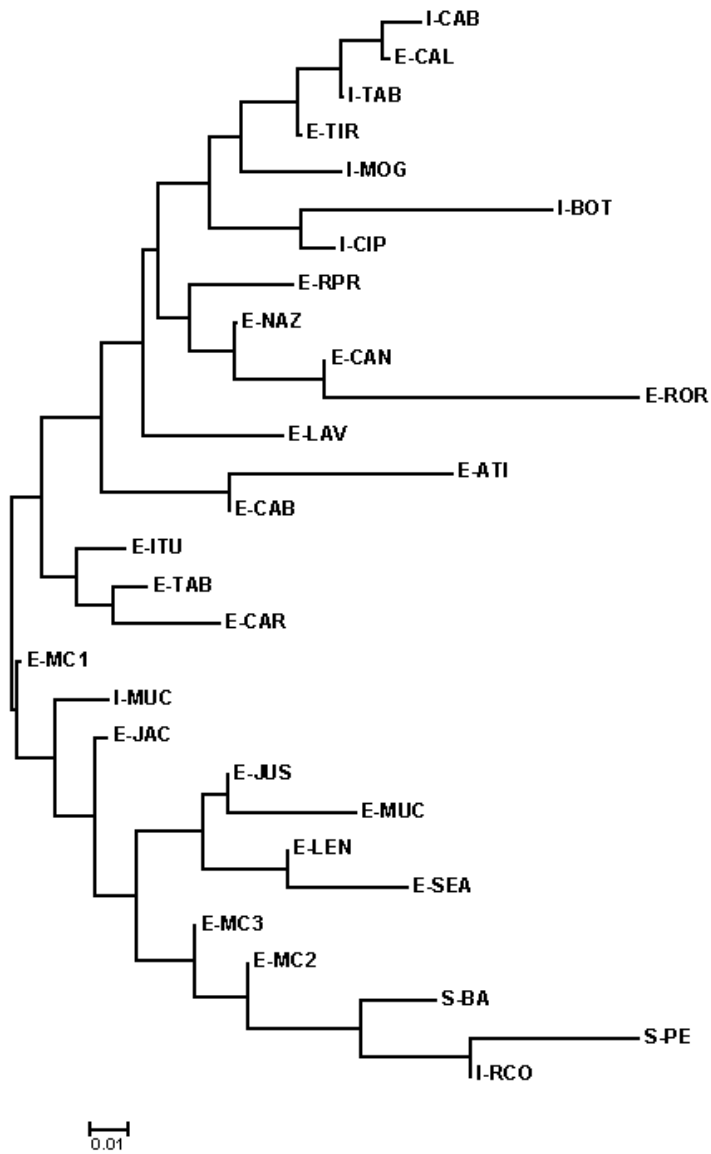

FIGURE 1. Neighbor-joining tree of 29 populations of *B. sanderianum* (S-) (two pops.), *B. involutum* (I-) (seven pops.) and *B. exaltatum* (E-) (20 pops.), based in nine allozymic loci and constructed using the matrix of Nei genetic distances (1978; unbiased genetic distance).

alleles of the locus MDH-1 is probably the main responsible factor for the separation of the two large groups of populations. The first group corresponds to the populations of the states of Bahia and Pernambuco, where the allele MDH-1 100 is most frequent (except for the population E-MC1 of Morro do Chapéu and I-MUC of Mucugê). The second is formed by the populations from São Paulo, Minas Gerais and Roraima States, where the allele 93 is the most frequent (Fig. 2) The genetic differentiation test F_{ST} based on *?* confirms the important participation of this locus in the structuring of the populations for the separation. Perhaps, hybridization events and early differentiation among the taxa have contributed to maintain the high genetic identity among populations, thus generating the observed reticulate pattern of clustering among different species (Fig. 1).

Morphological data suggest that *B. involutum* popula-

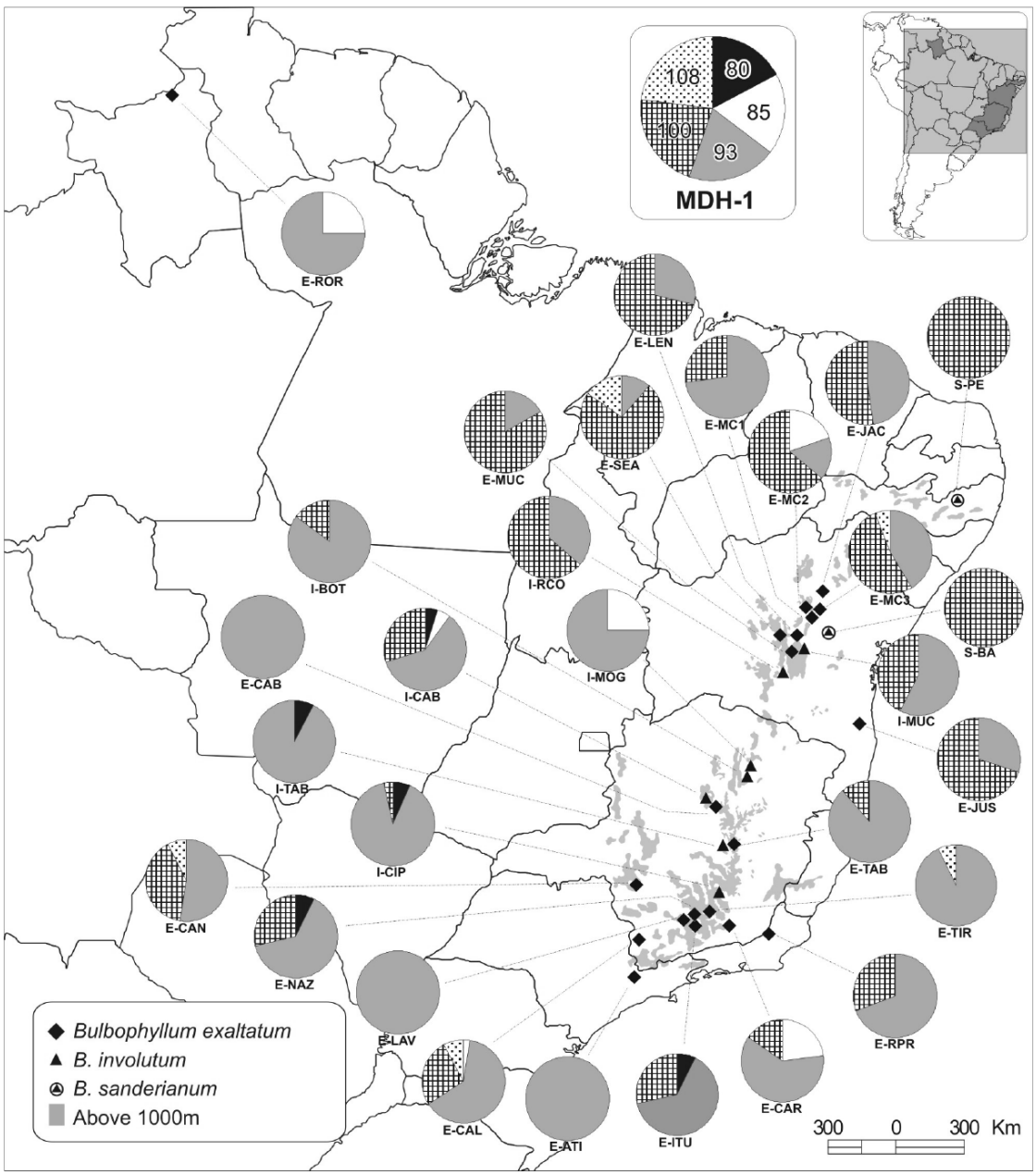

FIGURE 2. Graphic representation of the allele frequencies of the loci MDH-1 in populations of *B. exaltatum* (E-), *B. involutum* (I-) and *B. sanderianum* (S-) studied. Notice the inversion in the relative frequency of the alleles 93 and 100 among the populations from Minas Gerais and Bahia (except I-MUC e E-MC1).

tions in Minas Gerais stand out as a distinct taxon in relation to *B. exaltatum*. However, the populations of both taxa in Bahia State displayed lower differentiation (Fig. 3). In the first axis of the analysis (CVA) we observed more clearly the separation between the populations of *B. exaltatum* from Minas Gerais from Bahia and Roraima plus *B. involutum*, mainly for the smallest size of the lip and larger width of the dorsal sepal in the former. The second axis separates *B. exaltatum* from Bahia plus Roraima from *B. involu-*

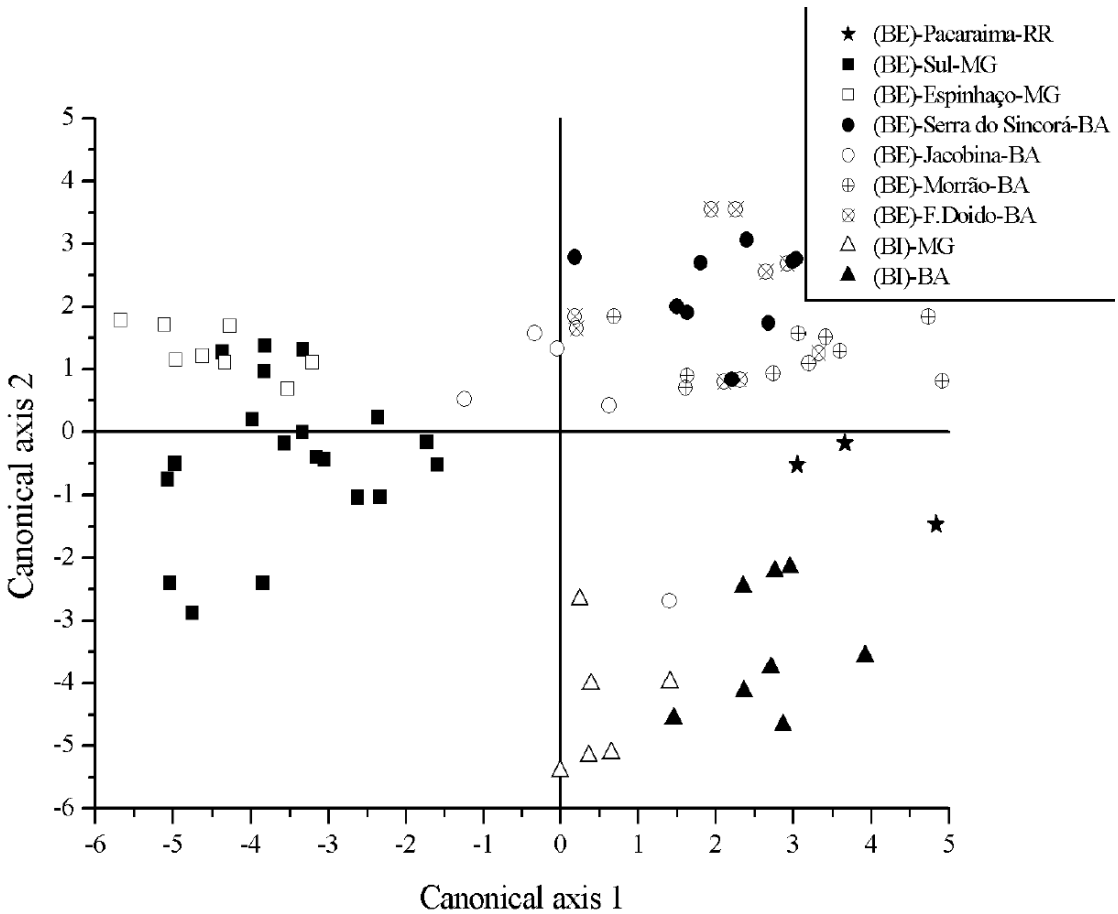

FIGURE 3. Graphic representation of the dispersion on the two first axes of the canonical analysis (CVA), based in 21 morphological characters, from individuals of nine groups of populations of *B. involutum* (BI) and *B. exaltatum* (BE) established *a priori* by geographical areas. Percentual of accumulated variation in the first two axes=71% (axis 1 = 47.3%; axis 2 = 23.7%).

tum. The canonical axes one and two accumulated respectively 47.3 and 23.7% of the variance.

Genetic and morphological data point out that the geographical barriers between Bahia and Minas Gerais, and the larger distance of the Roraima population suggest genetic and morphological differentiation between the populations from these States. The differentiation between the populations from Minas Gerais and the populations from Bahia apparently are related to the main separation of the Cadeia do Espinhaço in two portions. The northern portion is called Chapada Diamantina, and lies entirely in Bahia State, and the south includes the Planalto de Diamantina in Minas Gerais. This geographical separation is a north-south gap of 300 km with lowlands and has been considered as a strong geographical barrier to the migration of campo-rupestre plant species, with apparently great contribution in the differentiation of the plants in the campos rupestres from these areas (Giulietti & Pirani 1988, Harley 1988). Several other disjunctions affecting the geographical distribution in this vegetation has frequently been associated to the genetic differentiation of populations in several groups of plants (Borba *et al.* 2001, Jesus *et al.* 2001, Lambert *et al.* 2006).

Based on genetic, morphological, and reproductive biology of these and other *Bulbophyllum* species studied, we can conclude that all of the populations

considered *B. exaltatum* from Minas Gerais and São Paulo States should be treated taxonomically as a single entity, not divisible even in infraspecific categories. *Bulbophyllum exaltatum* is the oldest name to be applied to the populations from Minas Gerais, generally referred as *B. warminginum*, *B. ipanemense* or *B. geraense* in the literature, thus requiring new synonymies.

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LITERATURE CITED

Borba, E.L & Semir, J. 1998. Wind-assisted fly pollination on three species of *Bulbophyllum* (Orchidaceae) occurring in the Brazilian campos rupestres. *Lindleyana* 13: 201-218.

Borba, E.L, J.M. Felix, V.N. Solferini & J. Semir. 2001. Fly-pollinated *Pleurothallis* (Orchidaceae) species have high genetic variability: evidence from isozyme markers. American Journal of Botany 88: 419-428

Dressler, R.L. 1993. Phylogeny and classification of the orchid family. Portland: Dioscorides Press.

Giulietti, A.M & J.R Pirani. 1988. Patterns of geographic distribution of some plant species from the Espinhaço Range, Minas Gerais and Bahia, Brazil. *In*: Vanzolini P.E, W.R. Heyer (eds.). Proceedings of a Workshop on Neotropical Distribution Patterns. Rio de Janeiro: Academia Brasileira de Ciências, 36-69.

Harley, R.M. 1988. Evolution and distribution of *Eriope* (Labiatae) and its relatives in Brazil. In: Vanzolini, P.E, Heyer, W.R (eds.). Proceedings of a Workshop on Neotropical Distribution Patterns. Rio de Janeiro: Academia Brasileira de Ciências, 71-120.

Jesus, F.F, V.N. Solferini, J. Semir & P.I. Prado. 2001. Local genetic differentiation in *Proteopsis argentea* (Asteraceae), a perennial herb endemic in Brazil. Plant Systematics and Evolution 226: 59-68.

Lambert, S.M, E.L. Borba, M.C. Machado & S.C.S Andrade. 2006a. Allozyme diversity and morphometrics of *Melocactus paucispinus* (Cactaceae) and evidence for hybridization with *M. concinnus* in the Chapada Diamantina, North-eastern Brazil. Annals of Botany 97: 389-403.

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PROBLEMAS FITOSANITARIOS QUE AMENAZAN LA CONSERVACIÓN DE LAS ORQUÍDEAS EN COSTA RICA

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ABSTRACT: Orchids from private collections, nurseries and wild areas, located in different ecological regions of Costa Rica, were studied in order to know the pests and diseases affecting them. 16 insect and two mite genera were identified as pests on different orchids and 28 pathogens were found as causal agents of infectious diseases. The most frequent pests were: *Tenthecoris orchidearum, Stethobaris* sp., *Xylosandrus compactus, Pseudococcus longispinus, Diaspis boisduvalii* and *Tenuipalpus pacificus*. The main diseases found were caused by: *Phytophthora* spp., *Pythium* spp., *Fusariun oxysporum, Fusarium* spp., *Sclerotium rolfsii, Colletotrichum gloeosporioides, Sphenospora* spp., *Uredo* spp., *Cymbidium* Mosaic virus and *Odontoglossum* Ring Spot Virus. This results demonstrate the diversity of organisms producing sanitary problems on orchids in Costa Rica, and suggests the inclusion of pest and diseases management in strategies for orchid conservation.

KEY WORDS: orchid diseases, orchid pests, enfermedades, plagas, sanidad vegetal

Introducción

La conservación de las orquídeas debe ser una acción integral donde participen especialistas de diferentes disciplinas, sin olvidar la meta común de proteger esta noble familia de plantas. La pérdida de especies no es producto de un solo factor, es por el contrario, el efecto combinado de varios de ellos, particularmente los que son manipulados por el hombre. Dentro de estos debe destacarse la modificación que hemos hecho en el entorno biótico y abiótico, la extracción y comercialización ilegal de plantas, el mal manejo cultural de las orquídeas y los problemas sanitarios.

Las iniciativas para poner en marcha programas de conservación y rescate de especies, así como la protección de extensas regiones donde estas plantas viven y se reproducen libremente. Han hecho una función muy importante y se han obtenido avances significativos, en lo que a conservación se refiere. Sin embargo, algunas veces el resultado de todo ese esfuerzo y recursos puede verse amenazado por factores poco perceptibles en un inicio, pero devastadores a mediano o largo plazo, como son los problemas fitosanitarios que afectan las orquídeas.

Por lo general, las especies conservadas *in situ* no son tan amenazadas por plagas microbianas o de artrópodos, pues en la naturaleza existen controladores biológicos y diversas interacciones en el ecosistema que mantienen el problema bajo control, excepto cuando llegan organismos exóticos sin sus antagonistas. En las colecciones *ex situ* el panorama es diferente debido a que la intrincada red de interacciones, común en ecosistemas no alterados, está ausente o incompleta, las condiciones de crecimiento son artificiales, los genotipos son más homogéneos y hay una mayor manipulación tanto física como genética de las plantas. Esto hace que las plagas puedan encontrar un substrato muy propicio para desarrollarse y poner en peligro la sobrevivencia de especies muy selectas, escasas o ausentes en estado silvestre. Por lo tanto, sea cual fuere la modalidad de conservación siempre existe el peligro real o potencial de problemas fitosanitarios. Partiendo de esta premisa, la Escuela de Ciencias Agrarias de la Universidad Nacional, tomó por iniciativa propia el objetivo de estudiar los problemas sanitarios de las orquídeas en Costa Rica, con el fin de contribuir a la conservación de estas plantas en los diferentes sitios donde crecen cultivadas o en condición silvestre, como son las colecciones privadas, viveros, jardines botánicos, bosques cercanos a sitios de cultivo o áreas protegidas. Con este estudio se pretende establecer una plataforma de partida para proteger en una forma más integral las orquídeas de

Costa Rica y establecer los nexos necesarios con otros profesionales comprometidos con la conservación de las orquídeas.

Metodología de trabajo

Esta investigación dio inicio en el 2003 y concluirá en diciembre del 2006. Para su ejecución se consideró las diferentes regiones del país y se planificó una serie de visitas a cada una de ellas, iniciándose primero con el Pacífico Sur y Pacífico Central, luego se continuó con el Caribe y Zona Norte, para cerrar el proceso en el Pacífico Norte y el Valle Central. En cada región se contactó las diversas asociaciones regionales de orquideología, grupos de orquideófilos, productores comerciales y centros de investigación en este campo. Estos grupos o instituciones brindaron su ayuda en la ubicación de los sitios de visita y recorridos. A cada visita asistió un entomólogo y un fitopatólogo para hacer un reconocimiento de los problemas presentes, condiciones del sitio, historial sanitario y también para realizar la toma de muestras de plantas o partes de ellas con síntomas de problemas sanitarios. Los materiales recolectados fueron llevados a los respectivos laboratorios en la Universidad Nacional, para hacer los diagnósticos. En aquellos sitios donde habían orquídeas creciendo en condición silvestre o semisilvestre en fincas o bosques de los alrededores, también se tomaron muestras para estudio. En el laboratorio se realizaron los análisis rutinarios para organismos patógenos, insectos y ácaros y se levantó un registro de resultados e imágenes fotográficas. Cada vez que se concluyó el trabajo en una región o comunidad se brindó una conferencia sobre los resultados obtenidos y se brindaron las recomendaciones pertinentes para el manejo de esos problemas.

Resultados

Los patógenos observados hasta el momento en este estudio son 18 géneros de hongos, dos de Oomycetes, dos de Mixomycetes, cuatro bacterias, un alga y dos virus. Los hongos fitopatógenos identificados en diversos géneros de orquídeas corresponden a los siguientes:

Botrytis cinerea Pers.: Fr., agente causal de lesiones en flores de los siguientes géneros: *Cattleya, Epidendrum, Gongora, Guarianthe, Miltonia, Phalaenopsis, Peristeria, Pescatorea, Psychopsis, Stanhopea, Vanda* y *Xylobium*.

Capnodium sp. asociado a fumaginas en: *Dendrobium*, *Encyclia, Gongora*, *Maxilaria*, *Phalaenopsis*, *Pleurothallis*, *Rodriguezia*, *Stanhopea* y *Vanda*.

Cercospora spp. presente en manchas foliares de los géneros: *Acineta*, *Aspasia*, *Brassia*, *Calanthe, Catasetum*, *Cattleya*, *Coelogyne*, *Dendrobium, Dendrochilum, Encyclia*, *Epidendrum, Gongora, Huntleya, Lycaste*, *Maxilaria, Mormodes, Oncidium, Phaius*, *Pleurothallis*, *Rossioglossum*, *Sarcoglottis*, *Stanhopea* y *Trichopilia.*

Cercosporidium sp. causando manchas difusas en el follaje de: *Cattleya*, *Encyclia*, *Lycaste*, *Stanhopea* y *Trigonidium*.

Cladosporium sp., desarrollando fumagina en las inflorescencias de *Arundina graminifolia* (Don) Hochr.

Colletotrichum gloeosporioides (Penz.) Penz & Sacc [*Glomerella cingulata* (Stoneman) Spauld & Schrenk.], causando antracnosis en los siguientes géneros: *Acineta*, *Angraecum, Arundina*, *Aspasia, Brassavola, Brassia, Calanthe*, *Catasetum, Cattleya*, *Caularthron, Chysis*, *Cochleanthes, Coelogyne*, *Coryanthes*, *Cycnoches*, *Cymbidium, Dendrobium, Dracula*, *Dressleria*, *Encyclia, Epidendrum*, *Gongora, Guarianthe, Huntleya*, *Laelia*, *Lockhartia*, *Lycaste, Masdevallia, Maxilaria*, *Miltonia*, *Mormodes, Notylia*, *Oncidium, Paphiopedilum, Peristeria, Pescatorea, Phaius, Phalaenopsis, Pleurothallis, Polystachya, Psychopsis, Rossioglossum, Scaphyglottis*, *Schombugkia*, *Sobralia, Stanhopea, Trichopilia, Trigonidium*, *Vanda, Vanilla* y *Xylobium*.

Fusarium oxysporum Schlechtend.:Fr., como agente causal de marchitez en: *Bulbophyllum, Calanthe, Cattleya, Clowesia, Coelogyne*, *Cymbidium, Dendrobium, Encyclia*, *Epidendrum*, *Gongora, Guarianthe, Laelia, Lemboglossum*, *Miltonia*, *Mormodes, Oncidium*, *Phalaenopsis, Rodriguezia*, *Rossioglossum*, *Scaphyglottis*, *Vanilla* y *Xylobium*.

Fusarium spp., este patógeno fue aislado en diversas ocasiones a partir de pudriciones en raíces, rizomas y seudobulbos de géneros como: *Acineta*, *Brassia*, *Catasetum*, *Cattleya*, *Coelogyne*, *Dendrobium*,

Encyclia, Gongora, *Guarianthe*, *Laelia*, *Lycaste*, *Mormodes*, *Vanilla* y *Xylobium*.

Graphium sp., este hongo fue identificado en cultivos puros obtenidos a partir de manchas foliares en: *Cattleya*, *Cochleanthes*, *Coelogyne*, *Guarianthe*, *Lycaste* y *Oncidium*.

Guignardia sp., se observó como agente causal de manchas foliares en *Ascocentrum* y *Vanda*.

Lasiodiplodia (*Botryodiplodia*) sp., hongo asociado con diversas manchas en: *Brassia, Cattleya, Elleanthus*, *Gongora, Guarianthe*, *Maxilaria*, *Oncidium*, *Psychopsis, Sobralia*, *Stanhopea*, *Vanda* y *Vanilla.*

Meliola sp., agente causal del mildiú negro en: *Dendrobium*, *Dressleria*, *Schomburgkia* y *Vanilla*.

Mycoleptodiscus sp., causante de manchas foliares en los siguientes géneros: *Brassia*, *Coelogyne, Cyrtopodium*, *Elleanthus*, *Maxilaria*, *Oncidium, Phaius, Pleurothallis, Sobralia*, *Stanhopea* y *Xylobium.*

Phyllosticta sp., asociado a manchas foliares en: *Aspasia*, *Encyclia, Epidendrum* y *Vanilla*.

Sclerotium rolfsi Sacc., agente causal de pudriciones en la raíz de *Phalaenopsis* y base de la planta en *Spathoglottis*.

Sphenospora spp., causante de la roya en: *Catasetum*, *Encyclia*, *Epidendrum* y *Stanhopea*.

Schyzothirium sp., patógeno hallado en manchas foliares de los géneros: *Elleanthus*, *Oncidium*, *Pleurothallis*, *Sobralia* y *Stanhopea*.

Uredo sp. agente causal de royas en: *Brassavola*, *Cattleya*, *Encyclia*, *Epidendrum*, *Laelia*, *Pleurothallis* y *Trigonidium*.

Los restantes patógenos (Oomycetes, bacterias, Myxomycetes, algas y virus) identificados en diferentes localidades del país se presentan en el cuadro 1, con sus respectivos hospederos. De ese grupo destacan por su frecuencia los Oomycetes, causantes de la pudrición negra de plántulas y plantas adultas, la bacteria *Erwinia,* agente causal de pudriciones suaves y los virus que propician el desarrollo de síntomas como anillos cloróticos, anillos necróticos, estrías foliares y variegado de flores.

En el cuadro 2 se presentan los artrópodos determinados como causantes de daños en orquídeas cultivadas y silvestres. En términos generales puede señalarse que se logró observar cuatro coleópteros, un hemíptero, siete homópteros, un lepidóptero, un ortóptero, dos himenópteros y dos ácaros, asociados a daños en orquídeas de diversos géneros y colectadas en distintas partes del país.

Discusión

En Costa Rica hay una amplia diversidad de orquídeas, de la cual aún no se conoce la totalidad de especies (Morales 2005a, Morales 2005b), de igual manera, existe un número considerable de organismos que atacan las orquídeas, de los cuales tampoco se tiene un inventario completo en el país. Los resultados de esta investigación reflejan esa diversidad de organismos tanto autóctonos como exóticos, distribuidos en todas las localidades visitadas (Rivera & Corrales 2003, Corrales & Rivera 2003, Rivera & Corrales 2005). Por ser este el primer intento por censar los problemas fitosanitarios, es posible que haya más organismos en otros sitios y se requiera más años de trabajo, para completar la lista de patógenos y artrópodos potencialmente capaces de convertirse en plaga.

En las enfermedades de orquídeas determinadas hasta el momento, en el territorio nacional, se puede hablar de tres grandes categorías de enfermedades: letales, degenerativas y las de impacto reducido. En el primer grupo están las que liquidan la planta en un tiempo relativamente corto, las cuales se convierten en la principal amenaza para la conservación de especies muy valiosas. Los patógenos que pueden ubicarse en esa categoría son: *Phytophthora* spp., *Pythium* spp., *Erwinia* spp., *Fusarium* spp. y *S. rolfsii*. Los dos primeros son los agentes causales de la pudrición negra en rizomas y seudobulbos, su acción es muy rápida y en pocos días elimina un espécimen (Cárdenas 2003); La bacteria *Erwinia* causa pudriciones suaves en rizomas y seudobulbos, en ellos desintegra todos los tejidos blandos con rapidez; *S. rolfsii* y *Fusarium* spp. son causantes de pudriciones masivas del tejido radical y causan a corto plazo la muerte de las plantas afectadas, al limitarse la absorción de agua y minerales.

La segunda categoría incluye las enfermedades que eliminan el hospedero en forma paulatina y sostenida propiciando un decaimiento progresivo hasta causar la

CUADRO 1. Oomycetes, bacterias, Mixomycetes, algas y virus identificados en plantas enfermas de orquídeas procedentes de distintas zonas ecológicas de Costa Rica.

Patógeno	Nombre de los géneros de orquídeas hospederas
OOMYCETES	
Phytophthora spp.	*Cattleya, Cymbidium, Dendrobium, Dracula, Encyclia, Epidendrum, Guarianthe, Lockhartia, Lycaste, Masdevallia, Maxilaria, Oncidium, Rodriguezia, Scaphyglottis, Schomburgkia, Sobralia.*
Pythium spp.	*Cattleya, Coelogyne, Dendrobioum, Guarianthe, Masdevallia, Pescatorea, Phalaenopsis, Pleurothallis, Vanda.*
BACTERIAS	
Erwinia spp.	*Brassia, Calanthe, Catasetum, Cattleya, Coelogyne, Cyrtopodium, Dendrobium, Guarianthe, Lycaste, Maxilaria, Miltonia, Oncidium, Phaius, Phragmipedium, Schomburgkia, Stanhopea, Trichopilia, Vanda.*
Pantoea sp.	*Brassia, Catasetum, Cattleya, Epidendrum, Guarianthe, Laelia, Maxilaria, Oncidium, Pescatorea, Phalaenopsis.*
Xanthomonas sp.	*Guarianthe, Schomburgkia, Vanilla.*
Pseudomonas sp.	*Coelogyne, Phalaenopsis, Rossioglossum.*
MIXOMYCETES	
Physarum sp.	*Guarianthe, Oncidium, Trichopilia.*
Hemitrichia sp.	*Trichopilia.*
ALGAS	
Cephaleuros virescens Kunze	*Cattleya, Coelogyne, Elleanthus, Masdevallia, Oncidium, Sobralia.*
VIRUS	
ORSV-TMVO	*Acineta, Cattleya, Cochleanthes, Dendrobium, Encyclia, Epidendrum, Guarianthe, Schomburgkia, Sobralia, Trichopilia, Vanda.*
CyMV	*Cattleya, Cymbidium, Encyclia, Lycaste, Oncidium, Phaius, Trichopilia.*

muerte. En este grupo hay varios patógenos como: *C. gloeosporioides, F. oxysporum,* virus del mosaico del *Cymbidium* (CyMV), virus del anillado del *Odontoglossum* (ORSV) y varias royas. El hongo *C. gloeosporioides* es el agente causal de la antracnosis, la cual es considerada como la más frecuente en los trópicos (AOS 1995, Rivera 1998) y llega a ser una enfermedad degenerativa en plantas mal nutridas o en estado de abandono; *F. oxysporum* actúa como un patógeno vascular que lentamente bloquea la circulación de savia en la planta, por lo tanto, origina una serie de síntomas reflejos en hojas y tallos como deshidratación, cambios de color y enanismo, entre otros; los virus por sus características patogénicas tan particulares son un buen ejemplo de muerte lenta y prolongada en muchas especies de orquídeas al declinar el vigor de la planta por periodos largos o en forma recurrente; para algunos géneros de orquídeas las royas constituyen un problema serio al reducir el crecimiento y propiciar una condición precaria por años.

El último grupo lo conforman las enfermedades de impacto reducido, las cuales en condiciones normales de manejo, no comprometen la supervivencia del hospedero. Sin embargo, bajo estrés constante o estado de abandono, pueden afectar considerablemente la salud de la planta y aniquilarla. A esta categoría pertenece la mayor parte de las enfermedades observadas en este estudio y algunas de ellas son: las manchas foliares, manchas en la flor, así como lesiones en los seudobulbos y tallos.

Entre los artrópodos asociados a daños en orquídeas destacan por su importancia como plagas los siguientes: *Tenthecoris orchidearum* Reuter, *Stethobaris* sp., *Xylosandrus compactus* Eichhoff, *Pseudococus longispinus* Targione-Tozzetti, *Diaspis boisduvalii* Signoret y *Tenuipalpus pacificus* Baker. De todos ellos *T. orchidearum,* conocido entre los cultivadores como chinche rojo, es el más frecuente. Los daños los causan principalmente las ninfas que se ubican en el envés de las hojas, donde provocan una decoloración

CUADRO 2. Artrópodos causantes de daños en orquídeas identificados en distintas regiones de Costa Rica.

Identificación	Géneros de plantas hospedantes
COLEOPTERA: CURCULIONIDAE	
Stethobaroides sp.	*Catasetum, Cattleya, Coelogyne, Dressleria, Encyclia, Epidendrum, Guarianthe, Masdevallia, Maxilaria, Oncidium, Pleurothallis, Stelis, Schomburgkia.*
Stetobaris sp.	*Brassavola, Catasetum, Epidendrum, Schomburgkia, Vanilla.*
COLEOPTERA: SCOLYTIDAE	
Xylosanrdrus compactus Eichhoff	*Brassia, Guarianthe, Laelia.*
COLEOPTERA: MORDELLIDAE	
Mordillistema sp.	*Cattleya, Vanda.*
HEMIPTERA: MIRIDAE	
Tenhtecoris orchidearum Reuter	*Catasetum, Clowesia, Cattleya, Epidendrum, Encyclia, Gongora, Guarianthe, Masdevallia, Maxillaria,Oncidium, Restrepia, Rodriguezia, Stanhopea,Stelis,Vanda.*
HOMOPTERA: DIASPIDIDAE	
Diaspis boisduvalii Signoret, *Saisettia hemispherica* Walker, *Vinsonia stellifera* Westwood, *Chrysomphalus* sp.	*Cattleya, Calanthe, Cymbidium, Epidendrum, Elleanthus, Guarianthe, Laelia, Lycaste, Oncidium, Oerstedella, Phaius,Plalaenopsis, Schomburgkia.*
HOMOPTERA: PSEUDOCOCCIDAE	
Pseudococcus longispinus Targione-Tozzetti	*Encyclia, Cochleanthes, Catasetum, Oncidium, Phalaenopsis.*
HOMOPTERA: APHIDIDAE	
Afidos aún no identificados	*Brassavola, Cattleya, Encyclia, Epidendrum, Guarianthe, Phalaenopsis.*
Cerataphis orchidearum Westwood	*Acineta, Brassia, Cattleya, Encyclia, Epidendrum,Guarianthe, Laelia, Lockhartia, Oncidium, Isochilus.*
LEPIDOPTERA: NOCTUIDAE	
Polilla aún no identificada	*Brassia, Cattleya, Encyclia, Guarianthe, Laelia, Schomburgkia, Stanhopea.*
HYMENOPTERA: FORMICIDAE	
Atta sp.	*Dendrobium, Sobralia.*
HYMENOPTERA: EURYTOMIDAE	
Avispas agalladoras no identificadas	*Encyclia, Oerstedella.*
ORTOPTERA: TETIGONIDAE	
Idiarthron sp.	*Acineta, Brassavola, Brassia, Cattleya, Guarianthe, Miltonia, Mormodes, Phalaenopsis, Rossioglossum, Stanhopea, Trichopilia.*
ACARINA: TENUIPALPIDAE	
Tenuipalpus pacificus Baker	*Cymbidium, Dendrobium, Epidendrum, Phalaenopsis.*
Tetranichus sp.	*Dendrobium, Gongora, Phaius, Stanhopea.*

blanquecina visible en ambos lados de la hoja. Las orquídeas con lámina foliar más delgada o moderadamente gruesa, son las que presentan mayor intensidad del daño. Esta plaga se ha observado en distintas regiones del país durante todo el año, pero hay una tendencia hacia el incremento de la población en periodos de escasa precipitación. El segundo insecto más frecuente fue la escama *D. boisduvalii*, la cual se encontró causando severos daños especialmente en viveros bajo techo de plástico o vidrio. Sin embargo,

también se observó con menor frecuencia, en plantas ubicadas en sombreaderos o al aire libre.

Los restantes insectos aparecen con frecuencia pero como casos aislados, donde no se ha apreciado daños severos, lo cual no implica que sean de importancia secundaria, pues también pueden convertirse en plagas bajo determinadas condiciones.

Si se desea establecer un plan para proteger las orquídeas de este país, en forma integral y sostenible en el tiempo, es indispensable incluir aspectos fitosanitarios en él. Esta necesidad se hace aún más evidente al analizar los resultados de este trabajo, el cual muestra la diversidad de artrópodos y microorganismos como plagas reales o potenciales, que se convierten en una amenaza permanente para la conservación de las orquídeas en Costa Rica.

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LITERATURA CITADA

American Orchid Society. 1995. Orchid pests and diseases. Florida. Book Department Services. AOS. 88p.

Cárdenas, C. 2003. Estudio de la pudrición negra de las orquídeas causada por *Phytophthora* sp. en colecciones del Valle Central de Costa Rica. Lankesteriana 7: 179-180.

Corrales, G. & G. Rivera. 2003. Principales artrópodos asociados con colecciones y viveros de orquídeas en la región del Pacífico Sur de Costa Rica. V Congreso Nacional de Fitopatología. San José Costa Rica. 17 –1 9 de noviembre 2003. 54 pp.

Morales, J.F. 2005a. Orquídeas de Costa Rica. Vol. 1. Heredia, Costa Rica. Editorial INBio. 180 p.

Morales, J.F. 2005b. Orquídeas de Costa Rica. Vol. 2. Heredia, Costa Rica. Editorial INBio. 165 p.

Rivera, G. 1998. Orquídeas, generalidades y cultivo. Heredia, Costa Rica. Editorial Fundación UNA. 263 pp.

Rivera, G. & G. Corrales. 2003. Principales enfermedades fungosas diagnosticadas en colecciones y viveros de orquídeas en la región del Pacífico Sur de Costa Rica. V Congreso Nacional de Fitopatología. San José Costa Rica.17 –1 9 de noviembre 2003. 27pp.

RRivera, G. & G. Corrales. 2005. Enfermedades fungosas y bacterianas de las orquídeas cultivadas en distintas zonas ecológicas de Costa Rica. XLV Congreso Anual de la Sociedad Americana de Fitopatología- División Caribe. VI Congreso Nacional de Fitopatología. San José. 27 de junio-0 de julio del 2005. 88pp.

Salazar, W., G. Rivera & G. Corrales. 2005. Identificación de patógenos e insectos que afectan la sanidad de las orquídeas epífitas en poblaciones silvestres de la zona protectora Cerros de la Carpintera. XLV Congreso Anual de la Sociedad Americana de Fitopatología- División Caribe. VI Congreso Nacional de Fitopatología. San José. 27 de junio-0 de julio del 2005. 88 pp.

German Rivera Coto. Catedrático de la Universidad Nacional. Ha trabajado por 30 años como Fitopatólogo en programas de investigación, docencia y extensión universitaria. En los últimos 20 años ha orientado su trabajo hacia la capacitación en el cultivo de las orquídeas y la investigación de los problemas fitopatológicos de la familia Orchideaceae, particularmente sobre enfermedades fungosas. Es autor de un libro sobre cultivo de las orquídeas con énfasis en los problemas sanitarios, varios artículos de revistas y ponencias en congresos nacionales e internacionales.

Gilberto Corrales Moreira. Profesor jubilado de la Universidad Nacional y de la Universidad de Costa Rica, donde fue docente, investigador y extensionista en entomología agrícola. En los últimos años ha colaborado *ad honorem* en el proyecto de investigación: Identificación de los patógenos y artrópodos que afectan la sanidad de las orquídeas en poblaciones silvestres y cultivadas. Producto de su trabajo ha realizado varias publicaciones sobre plagas de orquídeas.

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USO DE COMPLEJOS COMERCIALES COMO SUSTITUTOS DE COMPONENTES DEL MEDIO DE CULTIVO EN LA PROPAGACION *IN VITRO* DE *LAELIA ANCEPS*

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PALABRAS CLAVE: propagación *in vitro*, germinación asimbiótica, fertilizantes, *Laelia anceps*, conservación, sales basales, carbohidratos

Las laelias son las orquídeas mexicanas más típicas que se han cultivado por siglos. *Laelia anceps* subsp. *anceps* habita bosques de encino cálidos en México, Guatemala y Honduras. Es una planta epífita de 25 a 50 cm de altura, con pseudobulbos elipsoide-ovoides con hoja solitaria y 2-3 flores grandes, usualmente de color rosa púrpura (Halbinger 1997). Debido a sus flores llamativas es sometida a una presión de colecta muy grande, además de la destrucción de su hábitat, lo cual está provocando la disminución acelerada de sus poblaciones. A pesar de que las orquídeas producen frutos con cientos hasta millones de semillas (Benzing 1981, Arditti & Arditti 1986) dependen para germinar en su hábitat de una asociación micorrízica (Stancato *et al.* 1998), de características específicas del árbol hospedero y de condiciones ambientales favorables (Madison 1977, Dressler 1981); por lo que solo un porcentaje extremadamente bajo alcanza la madurez; un ejemplo fue *Encyclia boothiana* (Lindl.) Dressler en Florida, cuya colonia de 29 adultos produjo solo una plántula en un año (Stenberg & Kane 1998). Una de las prácticas más adecuadas para salvaguardar las orquídeas es a través de la germinación asimbiótica *in vitro*, como lo estableció Knudson en 1922 (Hicks 2005), obteniendo miles de plantas, con una variabilidad genética mucho mayor que la obtenida por la micropropagación clonal (Stenberg & Kane 1998), lo cual es deseable en caso de una reintroducción a sus áreas naturales. Las sales basales utilizadas tradicionalmente en los medios de cultivo para la germinación y desarrollo *in vitro* de otras laelias y géneros emparentados son las de Knudson C (KC) para *L. purpurata* Lindl. & Paxton (Stancato *et al.* 1998), *L. albida* Bateman ex Lindl. (Santos *et al.* 2005), *E. boothiana* (Stenberg y Kane 1998); y las de Murashige y Skoog (MS) para *L. flava* Lindl. (Moraes *et al.* 2005) y *Epidendrum radicans* Pav. ex Lindl. (Pateli 2003). Las sales basales, así como la sacarosa grado analítico, tienen un costo elevado y son de difícil adquisición en zonas rurales de la mayoría de las entidades federativas de México, por tal razón resulta conveniente sustituirlos por fertilizantes inorgánicos y azúcares caseros para la germinación y desarrollo *in vitro* de *L. anceps* subsp. *anceps*, con el propósito de establecer un protocolo útil para su propagación y se aproveche en comunidades rurales que exploten esta orquídea a través de un manejo sustentable.

Metodología

MATERIAL BIOLÓGICO. Se colectaron tres cápsulas maduras dehiscentes de *L. anceps* subsp. *anceps*, para obtener semillas.

DESINFESTACIÓN Y SIEMBRA *IN VITRO*. Se colocaron aproximadamente 100 semillas en sobres de papel filtro para su desinfestación, con etanol al 70% durante 5 min y hipoclorito de sodio (NaOCl) al 0.55% por 10 min, y siembra sobre los medios de cultivo para germinación. Al registrar que más del 50% de las plántulas presentaron al menos dos raíces, se transfirieron a los medios de cultivo para el desarrollo de

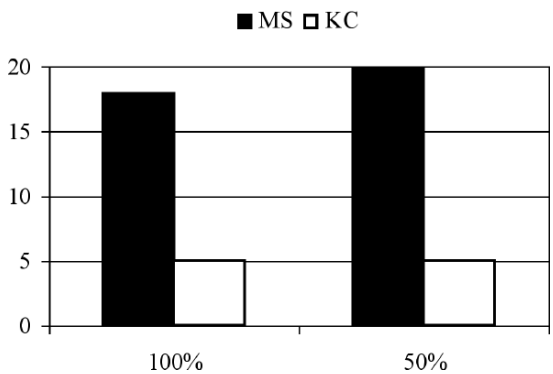

FIGURA 1. Desarrollo de plántulas a los 270 días de cultivo en medios de germinación.

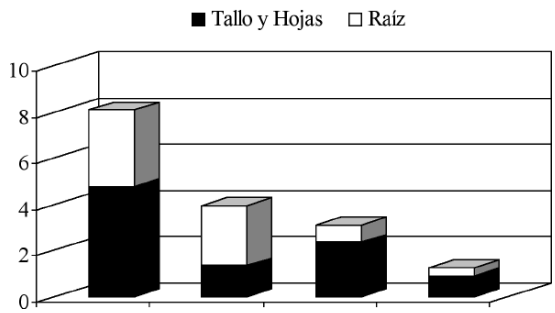

FIGURA 2. Peso fresco en gramos de plántulas a los 270 días de cultivo en medios de germinación.

plántulas con fertilizantes o con sustitutos de carbohidratos. Los cultivos se mantuvieron con un fotoperíodo de 16 horas de iluminación de 581 lux emitida por cuatro lámparas fluorescentes de 40W a 20º C.

PREPARACIÓN DE LOS MEDIOS DE CULTIVO. Se utilizaron para la germinación de las semillas las sales basales de MS (Sigma Chemical Cat. # M5524), adicionado con vitaminas y sacarosa grado analítico, y el medio de cultivo KC (Cat. # K4003). Se prepararon al 100% y 50% de la concentración de sales con diez repeticiones cada uno. Para el desarrollo de plántulas se prepararon medios de cultivo sustituyendo las sales basales con tres fertilizantes comerciales o tres sustitutos de carbohidratos. Los fertilizantes, Peters (P) (24-8-16), Floren (Fl) (10-15-5) y Folifértil (Fol) (20-30-10), fueron utilizados en tres concentraciones, al 100%, 50% y 25%, la primera concentración igualó el peso de las sales del medio MS utilizado como testigo. Los sustitutos de carbohidratos, azúcar refinada (AR), azúcar no refinada (ANR) y piloncillo (P) fueron adicionadas por separado al medio MS (testigo), en sustitución de la sacarosa grado analítico (S). Se utilizaron 20 plántulas o repeticiones para cada medio de cultivo. Todos los medios de cultivo fueron esterilizados a 20 lbs pulg^{-1} y 120° C durante 15 min. En los medios de cultivo para germinación se registraron a los 270 días el número y peso fresco de las plántulas germinadas. Las plántulas después de 100 días de cultivo, en el medio para su desarrollo, se registraron altura de la planta, longitud de raíz, número de raíces y plantas con pseudobulbos. A los datos obtenidos se les aplicó un análisis de varianza (ANDEVA), con el programa Statgraphics Plus 5.0, cuando el análisis fue no paramétrico se empleo la prueba de Krukal Wallis y el diagrama de cajas y alambres y cuando fue paramétrico se utilizó la prueba de intervalos de Tukey, para establecer en cual de los medios de cultivo se obtuvo mejor respuesta para la germinación y desarrollo de plántulas de *L. anceps* subsp. a*nceps*.

Resultados y Discusión

GERMINACIÓN. Después de 270 días de la siembra de las semillas de *L. anceps* subsp. *anceps* para su germinación se determinó que en los medios MS al 50 y 100% se desarrollaron una mayor cantidad de plántulas con dos o más raíces (Fig. 1). A pesar de no existir diferencias significativas entre ellas, las plántulas obtenidas en el medio MS 100% fueron más vigorosas, lo que se reflejó en su peso fresco (Fig. 2), ya que fue el doble del obtenido en el MS 50%. El medio MS, rico en sales minerales, favoreció el desarrollo de las plántulas de esta orquídea, incluso cuando se utilizó a la mitad de su concentración; a diferencia del medio KC, formulado especialmente para la germinación de orquídeas (Arditti & Ernst 1993). El desarrollo de plántulas vigorosas de *L. anceps* subsp. a*nceps* durante su germinación en MS, aunque lento, coincide con lo reportado por Stenberg y Kane (1998) para *E. boothiana*.

DESARROLLO DE PLÁNTULAS CON SUSTITUTOS DE SALES BASALES. Los medios de cultivo con fertilizantes que indujeron la mayor altura en las plantas, después de 100 días en cultivo, fueron P y Fl al 25% (Fig. 3), sin existir diferencias significativas entre ellos; mientras

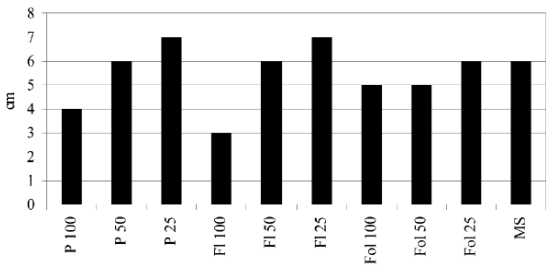

FIGURA 3. Longitud promedio de las plantas a los 100 días de cultivo en medio con sales basales o fertilizantes.

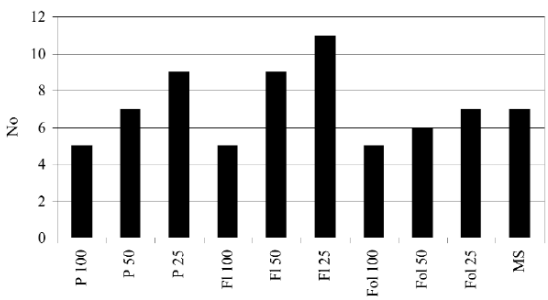

FIGURA 4. Raíces promedio por planta a los 100 días en medio con sales basales o fertilizantes.

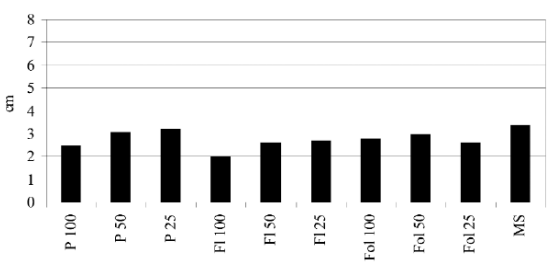

FIGURA 5. Longitud promedio de raíces a los 100 días en medio con sales basales o fertilizantes.

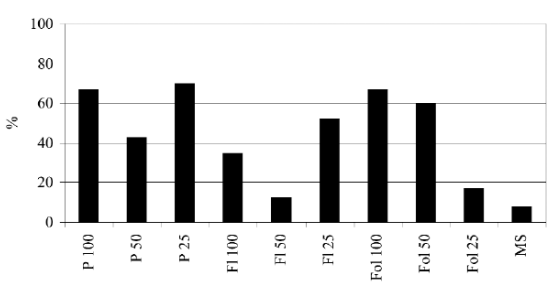

FIGURA 6. Plantas con pseudobulbos en medio con sales basales o fertilizantes a los 100 días de cultivo.

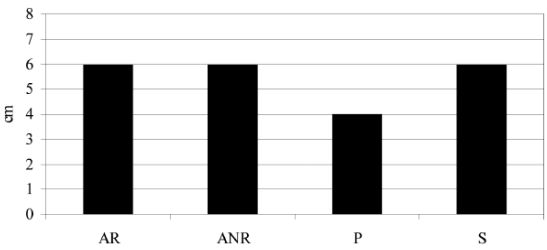

FIGURA 7. Longitud promedio de plantas en medio MS con sacarosa o sustitutos de carbohidratos a los 100 días de cultivo.

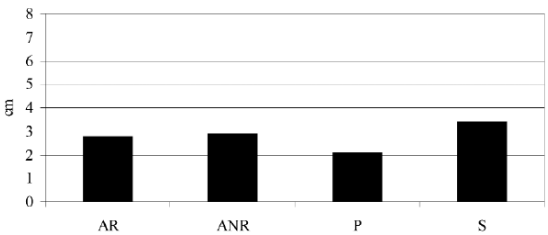

FIGURA 8. Longitud promedio de raíces en medio MS con sacarosa o sustitutos de carbohidratos a los 100 días de cultivo.

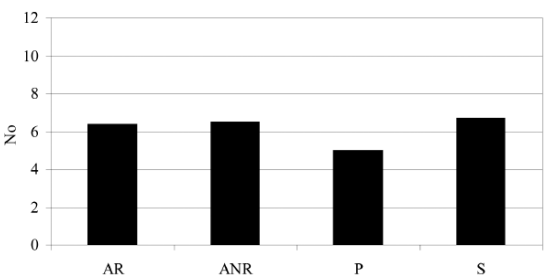

FIGURA 9. Raíces promedio por planta en medio MS con sacarosa o sustitutos de carbohidratos a los 100 días de cultivo.

que con Fl al 25% se generaron un mayor número promedio de raíces por planta (Fig. 4) y la longitud promedio de las raíces en todos los tratamientos osciló entre los 2 y 3.5 cm (Fig. 5). Una característica relevante del desarrollo de las plantas fue la diferenciación de los pseudobulbos, ésta varió significativamente entre los tratamientos, ya que al considerar el porcentaje de plantas que generaron pseudobulbos se obtuvo que 70% las desarrollaron con P al 25% de su concentración, disminuyendo un 3% de las plantas en P y Fol al 100%, 10% en Fol al 50% y 18% en Fl al 25%; lo que significa que en los tratamientos restantes se desarrollaron estas estructuras en menos del 50% de las plantas, resultando el menor porcentaje en

el medio MS (Figura 6). El fertilizante Peters es el único que contiene urea en su fórmula y posiblemente este elemento incrementó el contenido de nitrógeno total mejorando el desarrollo de las plantas.

DESARROLLO DE PLÁNTULAS CON SUSTITUTOS DE CARBOHIDRATOS. El medio de cultivo adicionado con P, como carbohidrato, resultó estadísticamente diferente ya que las plantas después de 100 días de cultivo se desarrollaron con una menor talla (Figura 7). En el medio con S las plantas desarrollaron raíces con mayor longitud (Figura 8), aun cuando no existieron diferencias significativas con los medios con AR y ANR; mientras que para la generación de raíces por planta (Figura 9) no existieron diferencias significativas, presentando un promedio de 5 a 7 por planta. El desarrollo de pseudobulbos fue despreciable estadísticamente, pues solo se desarrollaron en dos plantas de un total de 25, en el medio con S.

Conclusiones

El medio MS al 100 % favorece el desarrollo de plántulas vigorosas, con dos ó más raíces y mayor biomasa para la germinación. El uso de fertilizantes comerciales como sustitutos de las sales inorgánicas en el medio de cultivo favorece el desarrollo de plantas de *L. anceps* subsp. *anceps*. El fertilizante Peters al 25% promueve la generación de pseudobulbos y plantas con mayor tamaño. El uso de azúcar refinada o no refinada, como sustitutos de carbohidratos en el medio de cultivo, o de sacarosa grado analítico induce los mismos efectos en el desarrollo de las plantas. El uso del fertilizante Peters al 25% y azúcar no refinada como sustitutos de componentes del medio de cultivo favorecen el desarrollo vigoroso de plantas de *L. anceps* subsp. *anceps* en un periodo de 100 días. Estos sustitutos de medio de cultivo son económicos y de fácil adquisición.

LITERATURA CITADA

Arditti, J.M & R.E.Arditti. 1986. Some structural and physiological features which facilitate the survival orchids. *In*: Proceedings of the 11th World Orchid Conference: 102-105.

Arditti J. & R.Ernst. 1993. Micropropagation of Orchids. Wiley Interscience. USA. 682 pp

Benzing, D. 1981. Why is Orchidaceae so large, its seeds so small, and its seedlings mycomorphic? Selbyana 5(3-4):241-242

Dressler, R. 1981. The Orchids: Natural History and classification. Smithsonian Institution. USA. 332 pp.

Halbinger, F. & M.Soto 1997. Laelias of Mexico. Orquídea (Méx.) Vol. 15 México. 160 pp.

Hicks, A. 2005. Propagation of orchids from seeds. Orchid Rev. 113(1264):200-203

Madison, M. 1977. Vascular epiphytes: their systematic occurrence and salient features. Selbyana 2(1): 1-13

Moraes, L.M., R.T.Faria & F.L.Cuquel. 2005. Activated charcoal for *in vitro* propagation of Brazilian Orchids. ISHS Acta Horticulturae 683: V International Symposium on New Floricultural Crops.

Pateli, P.2003. Influence of *in vitro* culture medium on *Epidendrum radicans* seed germination, seedlings growth and *ex vitro* establishment. ISHS Acta Horticulturae 616: I International Symposium on Acclimatization and Establishment of Micropropagated Plants.

Santos, L., M.Martínez, J.Campos & E.Aguirre. 2005. *In vitro* propagation of *Laelia albida* (Orchidaceae) for conservation and ornamental purposes in Mexico. Hortscience 40(2):439-442

Sigma. 2006. Bioquímicos, reactivos y kits para investigación en ciencias de la vida. Catálogo 2006-2007. Sigma-Aldrich Co. USA

Stancato, G., E. Pereira & P.Mazzafera. 1998. Development and Germination of seeds of *Laelia purpurata* (Orchidaceae). Lindleyana 13(2): 97-100.

Stenberg, M. & M.Kane. 1998. In vitro Germination and greenhouse cultivation of *Encyclia boothiana* var. *erythronioides*, an endangered Florida Orchid. Lindleyana 13(2): 101-112.

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THE EFFECT OF THE LIGHT ENVIRONMENT ON POPULATION SIZE OF THE EPIPHYTIC HERB, *LEPANTHES RUPESTRIS* (ORCHIDACEAE)

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Introduction

The demographic dynamics of plant populations will depend on the relative role of density-dependent versus density-independent factors on population regulation. Density-independent factors (i.e. changes in climate, fire, hurricanes) will affect plant survival and reproduction within a population in a manner that is independent of the density of individuals. The amount of light that a plant receives is one such factor that will affect its mode of development, growth and reproduction independent of plant density. Life history traits may evolve when light requirements are a limiting factor in the survival of plant populations. Our research group has used the orchid *Lepanthes rupestris,* a common miniature orchid that inhabits riparian habitats mountain forests of Puerto Rico (Ackerman 1995, Tremblay 1997) as a model to understand the factors that affect the persistence of epiphytic orchids. In this study we were interested in determining if population size is affected by the amount of light perceived by the population.

Prior work on *L. rupestris* has studied demographic parameters and potential role of density-dependent effects in population regulation (Rivera-Gómez *et al.* 2006). At best, there was a positive (although very weak) relationship between the ratio of seedlings and juveniles to adults and population size, suggesting that some facilitation may be occurring. However, there was no relationship between the ratio of seedlings or juveniles to adults as population size regardless of substrate type (boulders or trees) suggesting that density dependence for population regulation in *L. rupestris* is likely to be rare. (Rivera-Gómez *et al.* 2006). Since density does not control the population size we sought to determine if the light environment affects population size. There are numerous studies on the effects of light environment on plant growth in orchids (Soontornchainaksaeng *et al*. 2001, Stancato *et al.* 2002), but not on population size.

We took advantage of a long-term census program of a metapopulation of *L. rupestris* in the Luquillo Mountains in Puerto Rico (Tremblay *et al.* 2006) to study the relationship between variation in light environment among plant patches (or subpopulations) and population size. As a preliminary study of the effect of the light environment on survivorship of populations we tested the following hypothesis. Under a theoretical light environment indicator distribution we expect that there should be an optimum light environment and that population size should reflect this distribution. Consequently there should be light environments where the indicator is in excess or below the required minimum, and thus populations size at these extremes should be generally small. A previous study investigating the relationship between light environments on growth rate of individuals has shown that growth rates in *L. rupestris* support this hypothesis (Fig. 1; Fernandez *et al.* 2003). Accordingly we should observe

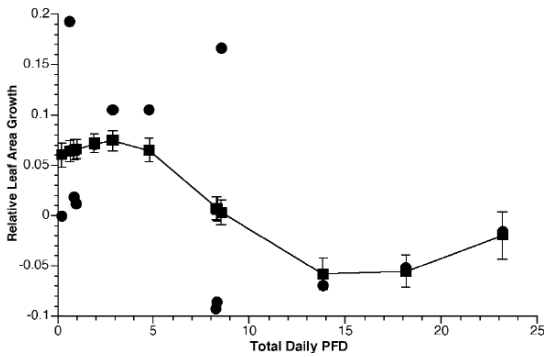

FIGURE 1: The cubic spline analysis of correlation between amount of light and growth rate is nonlinear and suggest maximum growth rate in the range of 1 to 5 Photon flux density (PFD), while higher PFD results in reduced growth rate. The data point are scattered below and above the best non-parametric fitness line suggesting that other environmental variables are likely to influence growth (Fernandez *et al*., 2003).

in an environment of too little or too much light small population sizes.

Plant species

Lepanthes rupestris is an orchid endemic to Puerto Rico that is commonly found along the riverbeds of the northwestern slopes of the Luquillo Mountains (Ackerman 1995, Tremblay 1997). It is common to find them on boulders, palms and trees (Fernandez *et al.* 2003). Plants are epiphytes and lithophytes (i.e., they anchor roots to the surfaces of trees or rocky boulders) and exhibit a maximum shoot height of 15 cm (Ackerman 1995). The populations of this species appear to behave as a metapopulation (Tremblay *et al.* 2006), which is defined as a set of subpopulations with asynchronous local dynamics occupying discrete patches (Hanski 1999, Hanski & Gaggiotti 2004).

Study site

The study was carried out along a 1000 m section of Quebrada Sonadora in the Luquillo Experimental Forest. Quebrada Sonadora is a steep first order tributary of the Espíritu Santo River, in northeastern Puerto Rico, latitude 18º 18'N, longitude 65º47'W (Tremblay *et al.* 2006). The section studied is between 400 and 500 m in elevation and runs through an area of secondary mature "Tabonuco" forest dominated by the tree species *Dacryodes excelsa* (Waide & Reagan 1996). Average annual precipitation is 3600 mm and somewhat seasonal, with a somewhat drier period between January and April (Brown *et al.* 1983, McDowell & Estrada Pinto 1988). Water discharge in these streams is highly variable and closely follows precipitation events (Johnson *et al.* 1998).

Materials and Methods

We examined a total of 191 subpopulations or plant patches. To estimate understorey light, we obtained hemispherical canopy photographs of the populations of Lepanthes rupestris, with a fisheye lens and digital camera. At each site photographs were taken above the highest concentration of individuals at about two inches above the plants. The objective was to capture the incoming light from the perspective of the population. Digital images were analyzed using HemiView Canopy Analysis Software (Version 2.1, Delta-T Devices, Cambridge, UK).

The light environment and canopy structure was characterized using the following indices: the direct site factor (DSF) and the indirect site factor (ISF), which represent the proportion of direct and diffuse radiation under the canopy relative to the levels outside the canopy, as well as the proportion of full sunlight penetrating the forest canopy (Global Site Factor, GSF). GSF combines direct radiation, by calculating the annual solar track, and diffuse radiation, based on a uniform overcast sky model (Clark 2003). In addition the proportion of visible sky (Visky) and the leaf area index (LAI) were estimated. LAI is the amount of projected leaf area in square meters above one-meter ground surface. We tested whether or not the distribution of this light environment indicators followed a normal distribution using a Shapiro-Wilkinson test.

We counted the number of individuals per each subpopulation in July 2006. We evaluated the relationship between light environment on five different life stages of the orchid as defined in Tremblay & Hutchings (2002) and Rivera-Gómez *et al.* (2006). The sum of the total number of all stages were correlated with the light indicator variables.

TABLE 1: Mean, standard error, median, 2.5 and 97.5 percentile of light environment indicators for 191 orchid populations. See methods and material for definition of indices.

Indicator	Mean	Standard Error	Median	Minimum 2.5 percentile	Maximum 97.5% percentile
Visky	0.082	0.003	0.073	0.023	0.179
ISF	0.123	0.004	0.108	0.025	0.296
DSF	0.156	0.007	0.130	0.020	0.414
GSF	0.152	0.007	0.127	0.020	0.401
LAI	2.93	0.053	2.81	1.95	4.94

It is expected that the variance in population size should increase as the quality of the light environment indicator reaches a maximum and that at the edge of this range population size variance should be smaller. Consequently we used a "Variability chart" to depict the variation in population size as a function of light environment indicator. All tests and analyses were conducted with the statistical program JMP (ver 6.0.0, SAS Institute Inc.). The square root of the population size was used for all analyses.

Results

The light environment indicators (Visky, DSF, ISF, GSF, LAI) show a non-normal distribution for all indices (Shapiro-Wilkinson W test for normality, all p's < 0.05). The amount of light perceived by the populations was generally low (Table 1) and in all cases skewed toward smaller values. For example, the proportion of visible sky above orchid populations is generally small with a mean of 8.2% (sd, 4.2%).

The light indicator indices do not predict any significant linear relationship to population size (all p's > 0.05; Table 2), except for leaf area index (LAI), where there is a positive relationship between total population size and leaf area index. Unfortunately the regression based on the LAI only explains 2% of the variation and consequently is inconsequential. Population size range and light indicators across all light variable index range showed in general a trend of smaller range of population size as the index of higher light indices increased in all light environment indicators except LAI (Fig. 2). However a pattern of the minimum threshold in the light indices on population size was not as clear, the two indices which might suggest this pattern are Visible sky (VisSky) and the indirect site factor (ISF), both of which show a reduced range in population size in the lower light indices categories.

Discussion

We found in general that the light environments where the populations of orchids are found are usually in the lower range of light index variables. Moreover, the only pattern that shows some consistency is a decrease in the variance in population size as light indices increase, suggesting a maximum threshold light environment for population growth rate. Clearly one may ask, how can we not have observed any stronger effect of the light environment on population size? On the other range of the light environment we found little evidence that limitation in light results in population size reduction. It is only logical for example that at some point too little light would bring about small populations. It is possible that the present extant populations are only in the environment where they growth more or less at their optimum. However, most populations were observed in only a subset of all light environments categories. It is likely that our sample sizes of too low and too high light environment were underrepresented and thus our ability to detect any effect was limited. An experimental design may be able to detect an effect

TABLE 2: Linear regression of light environment indicators as predictor of population size. Regression values, adjusted R squared and the Probability value for 191 orchid populations. See methods and material for definition of indices.

Indicator	Regression	Adj. R^2	P - value
Visky	6.26 - 5.32x	- 0.002	0.43
ISF	6.13- 2.54x	- 0.002	0.54
DSF	6.16 - 2.13x	-0.002	0.45
GSF	6.12- 2.24x	-0.002	0.45
LAI	3.39 + 0.82x	0.02	0.03

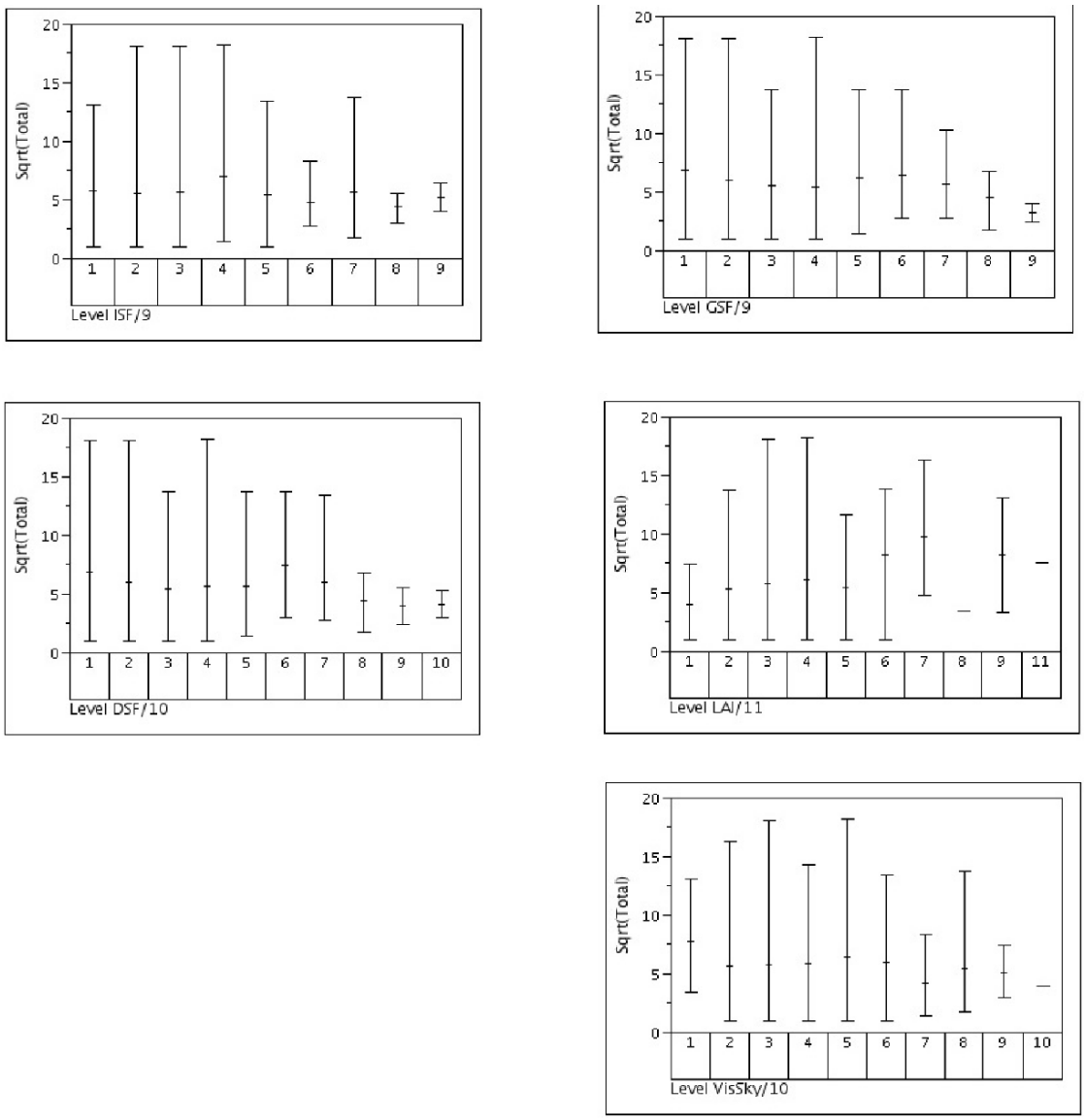

FIGURE 2: Variability Gage chart of the population size (sqrt) range and the varying categories of light indicators (Visky, DSF, ISF, GSF, LAI). The figure depict the range of the population size for low light levels (1) to high light levels (9-11) categories. The mean of each category is shown with a cross bar.

(if any) if conducted. To examine the real effect of exposure to light extremes in the population, there is a need for an experimental phase. In such experiment we can expose plants to extremes of light (a lot of light and practically no light) and observe the change in number (survival) and growth.

Another reason for the lack of difference between populations is the fact that these light measurements do not really measure the amount or quality of light perceived by plants, they are light indexes and they may not represent the actual light received by the plant. To make sure you are measuring photosynthetically important light and not relative light, light sensor could be installed in each population. In addition, light variability (measured as daily variance, for example), within a certain range of total radiation, may affect the population size instead of the actual total amount of light. The physiological basis for this

hypothesis may be found in the capacity of the photosynthetic apparatus of this species to respond to rapid light changes. Not only may light variability in the population affect population size and growth, but also light variability within a population.

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LITERATURE CITED

Ackerman. J.D. 1995. An orchid flora of Puerto Rico and the Virgin islands, Mem. New York Bot. Gard. (73): 1-208.

Brown, S., A.E. Lugo, S. Silander & L.H. Liegel. 1983. Research Opportunities at the Luquillo Experimental Station General Technical Report SO-44, New Orleans, LA: 128.

Clark, J. S., J. Mohan, M. Dietze & I. Ibanez. 2003. Coexistence: How to Identify Trophic Trade-offs. Ecology (84): 17–31.

Fernández, D. S., R.l. Tremblay, J.D. Ackerman, E. Rodríguez & N. López. 2003. Reproductive potential, growth rate and light environment in *Lepanthes rupestris* Stimson. Lankesteriana (7):73-76

Hanski, I.A. 1999. Metapopulation Ecology. Oxford University Press, New York.

Hanski, I.A. & O.E. Gaggiotti. 2004. Ecology, Genetics and Evolution of Metapopulations. Elsevier Academic Press, San Diego, California, USA.

Johnson, S.L., A.P. Covich, T. Crowl, A. Estrada-Pinto, J. Bithorn & W.A. Wursbaugh. 1998. Do seasonality and disturbance influence reproduction in freshwater atyind shrimp in headwater streams, Puerto Rico? Int. Vereiningung Theor. Limnol. Verh (26): 2076-2081.

McDowell, W. H & A. Estrada Pinto. 1988. Rainfall at the El Verde Field Station, 1964-1986. University of Puerto Rico CEER T-228.

Rivera-Gómez, N., R.L. Tremblay & E. Meléndez-Ackerman. 2006. Distribution of Life Cycle Stages in a Lithophytic and Epiphytic Orchid. Folia Goebotanica (41):107-120.

Soontornchainaksaeng, P., S. Chaicharoen, M. Sirijuntarut & M. Kruatrachue. 2001. In vitro Studies on the Effect of Light Intensity on Plant Growth of *Phaius tankervilliae* (Banks ex L' Herit.) Bl. and *Vanda coerulea* Griff. ScienceAsia (27): 233-237.

Stancato, G.C., P. Mqzzafera & M.S. Buckeridge. 2002. Effects of light stress on the growth of the epiphytic orchid *Cattleya forbesii* Lindl. X *Laelia tenebrosa* Rolfe. Revista Brasil. Bot., 2 (25): 229-235.

Tremblay, R.L. 1997. Distribution and dispersion patterns of individuals in nine species of *Lepanthes* (Orchidaceae). Biotropica (29): 38-45.

Tremblay, R. L. & M. J. Hutchings. 2003. Population dynamics in orchid conservation: a review of analytical methods based on the rare species *Lepanthes eltoroensis*. Orchid Conservation (eds.) K. Dixon, S. Kell, R. Barrett & P. Cribb. Natural History Publications (Borneo) Sdn. Bhd. Kota Kinabalu, Sabah, Malaysia. Pp. 183-204.

Tremblay, R.L., E. Meléndez-Ackerman & D. Kapan. 2006. Do epiphytic orchids behave as metapopulations? Evidence from colonization, extinction rates and asynchronous population dynamics. Biological Conservation (I29):70-81.

Waide, R.B. & D.P. Reagan. 1996. The rainforest setting. In: Reagan, D.P., Waide, R.B. (Eds.), The Food Web of a Tropical Rainforest. The University of Chicago Press, Chicago, IL, USA: 1-16.

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COMPARACIÓN DE LOS PROBLEMAS FITOSANITARIOS EN ORQUÍDEAS DE POBLACIONES SILVESTRES Y DE CULTIVO, COMO EVALUACIÓN DE RIESGOS DE PLAGAS O EPIDEMIAS

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PALABRAS CLAVE: orquídeas, plagas, entomología , enfermedades, patógenos, fitopatología

Introducción

Los ecosistemas naturales mantienen un autocontrol de poblaciones, que es afectada por factores exógenos. Mientras que en sistemas alterados se necesitan controladores exógenos para mantener un cierto tipo de equilibrio (p.e. prácticas culturales, uso de agroquímicos, subsidios de materia y energía, etc).
Las orquídeas que viven en condiciones alteradas suelen presentar problemas fitosanitarios, donde es necesario la intervención humana para asegurar la conservación de dichas especies, no así en medios naturales, pues existen mecanismos que controlan dichos problemas (Rivera 1999).

Aunque hay información del cultivo de las orquídeas que se venden comercialmente, son pocos los datos en este campo de especies silvestres. La dificultad en la medición de las demandas minerales y la desigualdad de requerimientos dificulta un estudio de necesidades nutricionales (Benzing 1990). Para el desarrollo pleno de las orquídeas es necesario de los factores propicios como son: luz, humedad, aireación, nutrición y control fitosanitario (Rivera 1998).

Las razones de documentar los diferentes problemas fitosanitarios en orquídeas son:

1. La poca información de interrelaciones biológicas entre orquídeas y sus antagonistas.
2. La destrucción de zonas silvestres, que propicia condiciones de desbalance y se afecta negativamente la conservación de muchas especies, entre ellas las orquídeas.
3. El traslado de orquídeas del estado natural a otro ambiente puede aumentar el riesgo de problemas fitosanitarios; facilitándose la diseminación de los mismos en viveros, colecciones privadas o jardines botánicos.
4. Estudios recientes revelan patógenos y artrópodos que afectan las orquídeas: AOS (1975), Rivera (1998), Cárdenas (2003), Amorosi (2004), Gutiérrez (2005), Corrales y Rivera (2003) y Rivera y Corrales (2003a, 2003b, 2005). Los problemas fitosanitarios documentados son variados pero no se conoce completamente las diferencias entre los ambientes naturales y los de cultivo.

En esta investigación se elabora una lista de agentes que afectan el desarrollo de las orquídeas en dos condiciones: natural (Zona Protectora Cerros de la Carpintera, Cartago) y artificial (Orquideario 25 de Mayo, Sabanilla de Montes de Oca). Se presentan dos poblaciones de orquídeas silvestres en condiciones climáticas similares pero en distintos ambientes.

Antecedentes

ZONA PROTECTORA CERROS DE LA CARPINTERA. La Zona Protectora Cerros de La Carpintera (ZPCC) fue creada mediante Decreto Ejecutivo 6112-A del 23 de junio de 1976. Mide cerca de 2.396 has y su manejo se rige por la Ley General Forestal No. 7575 del 13 de febrero de 1996. La ZPCC se localiza al sureste de San José. La cima de los cerros es divisoria de aguas entre la vertiente Atlántica y la vertiente Pacífica. Los tres picos más altos tienen alturas entre los 1.800 y 1.855 m. Otras alturas son el Cerro Quirazú (1.794 m), Alto Lima (1.651 m) y Alto Negro (1.747 m). El área de la ZPCC se distribuye entre los cantones de La Unión (974 has), Cartago (964 has), Desamparados (429 has) y Curridabat (28 has); donde

CUADRO 1.Uso de Tierra. Según el Sistema Nacional de Áreas de Conservación (SINAC), el uso de la tierra en la Zona Protectora.

Uso de tierra	Área	Porcentaje
Bosque primario	618 has	26%
Bosque secundario	194 has	8%
Charral	57 has	2%
Cultivos y pasto	769 has	32%
Suelo desnudo y ciudades	256 has	11%
Reforestación/recuperación	502 has	21%
Total	2396 has	100 %

el cantón de la Unión tiene la mayor riqueza natural. La precipitación anual es de 1.500 a 2.500 mm y la temperatura anual de 15 a 23° C (Ossenbach *et al* 2003) (Cuadro 1).

ORQUIDEARIO 25 DE MAYO. El Orquideario 25 de Mayo es una colección privada de orquídeas inscrita ante el MINAE como vivero artesanal desde el 8 de Julio del 2003 por medio de la resolución OSJ-SC-No V-001. Cuenta con más de 800 plantas en condiciones de cultivo con un plan manejo e identificación de especies. Está constituida por colectas a nivel nacional con el fin de facilitar los esfuerzos de conservación *in vivo*, y para investigación con la colaboración del Jardín Botánico Lankester. Está ubicado en el distrito de Sabanilla del cantón de Montes de Oca en la provincia de San José; a una altitud de 1.250 m.s.n.m con una precipitación anual de 1.900 mm y una temperatura anual de 20° C.

Marco teórico

Los problemas fitosanitarios son procesos naturales que afectan todos los cultivos. Son tan frecuentes y comunes tanto en la naturaleza como en cultivo (Rivera 1998).

En un ecosistema hay mecanismos reguladores de las poblaciones de organismos. En estado natural, las orquídeas forman parte de un sistema como ese, interactúan equilibradamente con otros seres vivos, así sus poblaciones sobreviven y se multiplican libremente. Cuando estas plantas se extraen de su ambiente natural y se colocan en plantaciones y colecciones, el balance natural se rompe y se incrementan algunos organismos antagonistas, cuya densidad puede afectar o deteriorar el cultivo (Cárdenas 2003). Además que se pueden presentar nuevas interrelaciones con otros organismos ajenos al hábitat original o la pérdida de otras interrelaciones afectando el funcionamiento de las plantas (Rivera 1998).

Entre las amenazas para la supervivencia de las orquídeas están las enfermedades infecciosas que pueden estar en forma silenciosa. La principal diseminación es el trasiego de materiales enfermos con infecciones latentes o con síntomas conspicuos (Rivera y Corrales 2005).“El predatorismo hacia las orquídeas es frecuente en los ecosistemas naturales y puede convertirse en plaga cuando se trata de plantas cultivadas” (Rivera 1998).

Las orquídeas en su hábitat natural tienen un excelente balance para desarrollarse a plenitud. Pero el mantenimiento de las mismas en colección o vivero requiere de un plan de manejo, así como una atención constante. Para que las plantas no se deterioren se necesita del tiempo, el conocimiento o los recursos apropiados.

Metodología

PROCEDIMIENTOS. Se recolectaron orquídeas que presentaron problemas fitosanitarios de las dos zonas de estudio. Las muestras recolectadas fueron empacadas, etiquetadas y llevadas al laboratorio para el análisis respectivo.

ENFERMEDADES. Se recolectaron plantas completas o secciones de las mismas, según el grado de la enfermedad. En el Laboratorio de Fitopatología de la Escuela de Ciencias Agrarias (ECA) de la UNA se realizó el proceso de diagnóstico e identificación, usando los procedimientos tradicionales según las técnicas correspondientes al tipo de patógeno. En el caso de plantas con virus no se aplicaron las técnicas moleculares, solo se describieron los síntomas correspondientes.

INSECTOS. Se recolectaron e identificaron aquellos individuos asociados al daño evidente en la orquídea. En el caso de insectos inmaduros se recolectó la planta completa para desarrollar las formas adultas en el laboratorio de Entomología de la ECA. Si no se presentó el agente causal de los daños se especuló sobre el posible causante.

Resultados

ENFERMEDADES

Antracnosis. Esta es la enfermedad más común en las orquídeas cultivadas en los trópicos. Se encontró ata-

cando hojas, flores, seudobulbos y brotes jóvenes. En las hojas produjo manchas ovaladas, circulares o de forma irregular, color café oscuro, negras o grisáceas. En hojas carnosas se observaron hundidas y con un borde bien definido. Las hojas delgadas muestran lesiones de color y forma similar con un hundimiento leve o ausente. En estados avanzados se observaron acérvulos organizados en anillos concéntricos o líneas curvas. En los seudobulbos las lesiones fueron ovaladas o irregulares, con un hundimiento muy marcado y colores que van de café rojizo a negro (según la especie). El agente causal de la enfermedad fue *Glomerella* sp. (teliomorfo) o *Colletotrichum* sp. (amorfo, forma más frecuente).

Marchitez. Es una enfermedad vascular, que presentó pudriciones secas en las raíces y rizomas de las orquídeas, asociadas a coloraciones púrpura o rosa en el rizoma. Se identificó por un decaimiento de la planta, coloración verde claro o amarillenta de los seudobulbos y acucharamiento de las hojas (principalmente aquellas de tipo carnosas). Es causada por *Fusarium* spp.

Pudrición negra. Se presentó en todas las partes de la planta. En follaje causó manchas negras o café oscuro de consistencia suave, las lesiones avanzadas cubrieron la hoja e inclusive las partes basales (tallos, seudobulbos, rizomas y raíces). "Es causada por *Phytophthora* spp. y *Pythium* spp" (Rivera 1998). Esta enfermedad se favorece por condiciones de mucha humedad, poca aireación y poca luminosidad.

Cercosporiosis. Inicia con pequeños puntos amarillos en la superficie de la hoja, luego se hacen visibles en la parte superior. Presentó patrones como parchones con clorosis o puntos marrones esparcidos a lo largo de la hoja. En estado avanzado las manchas coalescen deteriorando gran parte de las hojas. El patógeno fue *Cercospora* spp.

Roya. Esta enfermedad es poco frecuente en orquideários. Se identificó por pústulas generalmente en el envés de la hoja. Solamente se encontró ocasionado por *Uredo* sp.

Patógenos de suelo. Los patógenos identificados fueron: *Pythium* sp. y *Rhizoctonia* spp. y *Verticilium* sp. Se presentaron causando necrosis del tallo a nivel cortical.

Enfermedades causadas por bacterias. En las hojas se produjeron manchas negras con un halo de apariencia acuosa o aceitosa; mientras que en seudobulbos fueron pudriciones suaves generalmente con líquidos de olor desagradable. La bacteria asociada fue del género *Erwinia*.

Enfermedades causadas por algas. Es una enfermedad de tipo cosmética. Inicia con manchas negras en el haz de las hojas, luego se forman motas amarillas. Se produjo por *Cephaleurus virescens* Kunze.

*Enfermedades causadas por Virus. "*Los síntomas son poco seguros para dar un buen diagnóstico, varían mucho aún en un mismo hospedero" (Rivera 1998). Hay gran cantidad de investigación de virus en orquídeas (Pupulin, comentarios personales 2005), esto permite que "se puedan asociar ciertos síntomas a la conclusión de que sean provocados por virus sin necesidad de efectuar pruebas biomoleculares" (Rivera, Comentarios personales 2005). Los síntomas fueron: clorosis, mosaicos, anillos necróticos. En otros casos presentaron forma ojival formada por manchas necróticas o cloróticas.Las orquídeas que presentaron síntomas asociados a presencia de virus se encontraron solamente en el Orquideario 25 de Mayo.

Quemaduras de sol. Es una enfermedad abiótica por sobreexposición de la planta a la luz solar que deteriora los tejidos, causando: amarillamiento generalizado, crecimiento pobre o quemaduras. Los síntomas fueron quemaduras o edemas en hojas y seudobulbos. Inicia mostrando áreas verde claro con bordes indefinidos; luego cambian a color blanco-plateado y finalmente se tornan café o negro. "Este daño es cosmético, pero permite la entrada de patógenos" (Rivera 1998) El resumen de las enfermedades se presenta en el Cuadro 2.

INSECTOS

Defoliación sin presencia del agente causal. Se presentaron hojas y seudobulbos de orquídeas con diferentes tipos de daños mecánicos posiblemente ocasionados por insectos herbívoros generalistas: grillos (fam: Gryllidae), chapulines (fam: Acrinidae), larvas de mariposas (Lepidópteros), picudos (fam: Curculionidae) o crisomélidos (fam: Chrysomelidae).

Defoliación con presencia del agente causal. Fueron defoliaciones en hojas y flores causados por larvas de mariposas, crisomélidos y picudos.

CUADRO 2. Enfermedades identificadas.

Nombre de la enfermedad	Género de orquídeas del Orquideario 25 de Mayo	Género de orquídeas del ZPCC
Antracnosis	*Acineta*, *Cochleantes*, *Elleanthus*, *Epidendrum*, *Guarianthe*, *Huntleya*, *Kefersteinia*, *Laelia*, *Lockhartia*, *Maxillaria*, *Octomeria*, *Oncidium*, *Pescatorea*, *Pleurothallis*, *Prosthechea*, *Rossioglossum*, *Sobralia*, *Stelis*, *Stenorrhynchus*, *Tricopilia*, *Trigonidium*, *Vanilla*	*Elleanthus*, *Epidendrum*, *Maxillaria*, *Oerstedella*, *Oncidium*, *Pleurothallis*, *Prosthechea*, *Schaphyglotis*, *Sobralia, Stelis*, *Warczewiczella*, *Xylobium*
Marchitamiento	*Brassia*, *Encyclia*, *Guarianthe*, *Kefersteinia*, *Laelia*, *Maxillaria*, *Oncidium*, *Osmoglossum*	*Maxillaria*, *Prosthechea*
Pudrición negra	*Guarianthe*, *Rossioglossum*	*Prosthechea*
Cercosporiosis	*Maxillaria*, *Stelis*, *Trigonidium*	*Epidendrum radicans*
Roya	No se presentó	*Pleurothallis*, *Maxillaria*
Patógenos de suelo	No se presentó	*Prosthechea, Epidendrum, Stelis, Lepanthes*
Bacteriosis	*Brassia*, *Coelipsis*, *Eriposis*, *Hexisea*, *Maxillaria*, *Pleurothallis*, *Prosthechea livida*, *Xylobium*	*Maxillaria*, *Prosthechea*, *Stelis*
C. virescens	*Sobralia*, *Stelis*	No se presentó
Síntomas asociados a virus	*Cymbidium*, *Oncidium*, *Stanhopea*, *Tricopilia*	No se presentó
Quemaduras de sol	*Elleanthus*, *Guarianthe*, *Laelia*, *Maxillaria*, *Oncidium*, *Pleurothallis*, *Stelis*, *Vanilla*	*Maxillaria*, *Pleurothallis*, *Sobralia*

Polilla harinosa. Los daños por este insecto se manifestaron por un decaimiento de la planta y poco crecimiento. Las larvas de este insecto comen las raíces y con cierta frecuencia los seudobulbos. Los signos fueron los excrementos negros y grises presentes en el sustrato. Son larvas de color gris o café rojizo que forman un capullo con una seda blanca muy resistente y envueltas con las mismas excretas del insecto.

Minas y Galerías. Se presentaron de varias formas y tamaños, principalmente en hojas carnosas: variedad de serpentinas y minas regulares. Los insectos que se encontraron causando daños fueron especies de Diptera, Lepidoptera y Coleoptera (Curculionidae). Las galerías fueron de dos formas: agujeros dispuestos a lo largo de las hojas (sin un patrón definido) y agujeros a lo largo de la vena central de las hojas. Las minas y galerías inactivas pudieron ser ocasionadas por los antes mencionados o por Hymenopteros.

Agallas en raíces. Se presentaron en las puntas de las raíces, por oviposiciones de picudos y un euritomido (Orden Hymenoptera), causando anomalías en el crecimiento celular.

Hojas con pinchaduras. Fueron lesiones hechas por chinches u otros insectos que se alimentan de savia.

Escamas. Las escamas se presentaron en hojas, seudobulbos e inflorescencias. Al nutrirse de la savia de las plantas causaron coloración amarillenta o depresiones del tejido.

Áfidos Se presentaron en inflorescencias, nuevos brotes y algunas hojas adultas, succionando savia de los tejidos. El resumen de las enfermedades se presenta en el Cuadro 3.

Conclusiones

En los dos ambientes estudiados se presentaron con mayor frecuencia antracnosis y defoliación por insectos masticadores. Sin embargo fueron más frecuentes los problemas fitosanitarios en el Orquideario 25 de Mayo que en la ZPCC; posiblemente por condición de desequilibrio ambiental o por mayor hacinamiento en estado de cultivo.

En la ZPCC se observó una mayor concentra-

CUADRO 3 Daños provocados por insectos.

Daño	Género de orquídeas del Orquideario 25 de Mayo	Género de orquídeas del ZPCC
Defoliaciones sin presencia del agente causal	*Epidendrum*, *Oerstedella*, *Oncidium*, *Pleurothalis*	*Elleanthus*, *Encyclia*, *Epidendrum*., *Maxillaria*, *Oerstedella*, *Ornithocephalus*, *Pleurothallis*, *Prosthechea*, *Schaphyglotis*, *Warczewiczella*, *Xylobium*
Curculionidos	*Oerstedella*, *Pleurothallis*	*Epidendrum**, *Oerstedella**, *Pleurothallis**, *Stelis**
Chrysomelidos	*Epidendrum*	*Epidendrum*, Pleurothallis**
Polilla harinosa	*Brassia* * , *Maxillaria*, *Oncidium*, *Pleurothallis*	No se presentó
Minas	*Maxillaria*	*Epidendrum*, *Pleurothallis*, *Stelis*
Galerías*	*Arpophyllum*, *Encyclia*, *Prosthechea*, *Vanilla*	*Encyclia*, *Epidendrum*, *Pleurothallis*, *Schaphyglottis*, *Stelis*
Afidos	*Encyclia*, *Oncidium*, *Oerstedella*	*Oerstedella*
Escamas	*Elleanthus*, *Guarianthe*, *Laelia*, *Oncidium*, *Pescatorea*, *Schaphyglottis*	*Maxillaria*
Hojas con pinchaduras*	*Laelia*, *Pleurothallis*, *Prosthecea*	*Oerstedella*
Agallas en raíces	*Arpophyllum*	*Epidendrum*, *Oerstedella*

ción de problemas fitosanitarios en zonas con alteraciones o procesos de equilibrio ecológico; pero en zonas de Bosque Primario no hubo problemas sanitarios importantes.

La presencia de enfermedades latentes o insectos potencialmente plagas, son uno de los problemas que considero de suma importancia, en el marco de la manipulación de orquídeas tanto de condiciones de cultivo como de zonas naturales.

Para efectos de la conservación de orquídeas se debe seguir la investigación de problemas fitosanitarios; ya que hay diversidad de agentes que pondrían en riesgo el desarrollo pleno de estas plantas. Entre tanto se favorezca el deterioro de los ecosistemas o se de un mal manejo a colecciones *in vivo*, se estaría amenazando la biodiversidad en general. Los problemas fitosanitarios en orquídeas llegan a sumarse a la lista de peligros que directa o indirectamente ponen en riesgo la diversidad de esta familia, como son: la deforestación, trasiego ilegal de plantas, contaminación ambiental y erosión genética.

Como consideración final es apropiado llamar la atención a todo aquel interesado en el mundo de las orquídeas, donde hay que tener muy claro el manejo apropiado, así como el conocimiento para mantener y cuidar esta familia.

Este estudio podría servir de herramienta para futuras investigaciones sobre otras interrelaciones antagónicas.

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LITERATURA CITADA

American Orchid Society. 1975. Handbook on Orchid Pests and Diseases. AOS. Inc. Cambridge, Massachusets, U.S.A. 113 p.

Amorosi Mellado, R. 2004. Orquídeas Así hice mi vivero. Reglas prácticas de cultivo, desarrollo y mantenimiento de orquídeas. San José Costa Rica. Sociedad Editora Alquimia 2000. 63 p.

Benzing, D. 1990. Vascular epiphytes. New York, USA. Cambridge University Press 354 p.

Cárdenas Briceño, C. 2003. Diagnóstico de las enfermedades que afectan las orquídeas cultivadas en el Valle Central de Costa Rica. Heredia, Costa Rica. Escuela de Ciencia Agrarias, UNA. 165 p

Corrales, G. & G. Rivera. 2003. Principales artrópodos asociados con colecciones y viveros de orquídeas en la región del pacífico sur de Costa Rica. Memoria de Congreso Alianza Tecnológica para la Agricultura con Calidad. San José CR. pag. 54.

Gutiérrez, C. 2005. Diferencias de patotipos del hongo Colletotrichum gloesporioides, que ataca en los invernaderos comerciales y en un Jardín Botánico en el Valle Central de Costa Rica. Escuela de Ciencias Agrarias. Heredia. C.R. 49 p

Ossenbach C., M. Ossenbach & F. Pupulin. 2003. Catalogo Preliminar Anotado de las Orchidaceae de la Zona Protectora Cerros de la Carpintera. Orquideario 25 de mayo, San José Costa Rica. 41 p

Rivera Coto, G. 1998. Orquídeas Generalidades y Cultivo. Heredia, Costa Rica. Editorial Fundación UNA. 266 p.

Rivera Coto, G. 1999. Conceptos Introducción a la fitopatología. San José, Costa Rica. Editorial Universidad Estatal a Distancia. 308 p.

Rivera, G. & G. Corrales. 2003a. Principales enfermedades fungosas diagnosticadas en colecciones y viveros de orquídeas en la región del pacífico sur de Costa Rica. Memoria de Congreso Alianza Tecnológica para la Agricultura con Calidad. San José CR. pag. 27.

Rivera, G. & G. Corrales. 2003b. Principales enfermedades de orquídeas causadas por oomycetes, bacterias y algas en colecciones y viveros en la región del pacífico sur de Costa Rica. Memoria de Congreso Alianza Tecnológica para la Agricultura con Calidad. San José CR. pag. 28

Rivera, G. & G. Corrales. 2005. Estudio de las enfermedades que afectan las orquídeas en Costa Rica. Revista Ticorquídeas.1(1):13-14.

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TRADITIONAL USE AND CONSERVATION OF THE "CALAVERITA" *LAELIA ANCEPS* SUBSP. *DAWSONII* F. *CHILAPENSIS* SOTO-ARENAS AT CHILAPA GUERRERO MÉXICO

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Mexico lodges a heritage of traditional knowledge on use, management and conservation of a great variety of genetic resources tie to its culture and idiosyncrasy (Davis *et al*. 1994; Toledo 1991). That heritage is protected in the memory of the indigenous and rural villages of the country, which by means of the daily practice and oral transmission of their customs, have allowed several species to survive, among them some orchids of the genus *Laelia* (Halbinger and Soto 1997, Toledo 1991).

Considering that *Laelia anceps subsp. dawsonii f. chilapensis* is one of the most beautiful mexican orchids, and that around it exists an experience of traditional appropriation in some indigenous and rural communities, reason why it is well-known locally like "calaverita", this work had as objective to study the local strategy of use and conservation of the Calaverita (*Laelia anceps subsp. dawsonii f. chilapensis*) and its floral morphological variation in the region of Chilapa Guerrero, Mexico.

The study was carried out through methodologies of qualitative and quantitative order: a) Oral history for reconstruction of the context in which the traditional use system develops and the collective memory that validates the practices (Of Garay 1994). For this point they were considered two women of 84 and 76 years old as key informants, both women were determined and recognized by the own community as authorities on the calaverita knowledge. b) Lip morphometric analysis, to represent variation within the clones of the chilapensis form. It included the dissection and placement below a glass slide for drawing of 145 lip samples, measurement of lengths of 32 lines in a truss network drawn up to the interior of lip, logarithmic transformation of the measurements to consider the proportion, the generation of a matrix of correlations among the 32 variables, and a Principal Component Analysis (PCA) with the statistical package SAS (SAS 1995, Catling 1990).

The results show that the presence of calaveritas in the community, depends on the interrelations among; the presence of the backyard or *solar* structure, the woman's role and the use value as predominant form of assessment among the inhabitants of the Chilapa region. The traditional backyard or *solar* are spaces bordering to the house where it keeps a great diversity of vegetal and animal resources destined for family self-consumption. Since pre-hispanic times these sites have worked as storage and transmission centers of traditions, customs and values that determine the traditional life form that prevails in the Chilapa region; through the family at a first instance and specially through the woman. The feminine performance is fundamental in the execution of the management practices and the values that maintain and conserve the calaverita, since in the women resides the possession of the knowledge and its transmission. The transmission process is made by two ways; the first way from mother to daughter. The mother, who is in charge of all the activities that are made in the backyard or *solar*, teaches to its daughters and involves them with the practices, particularly with those related to the religious and

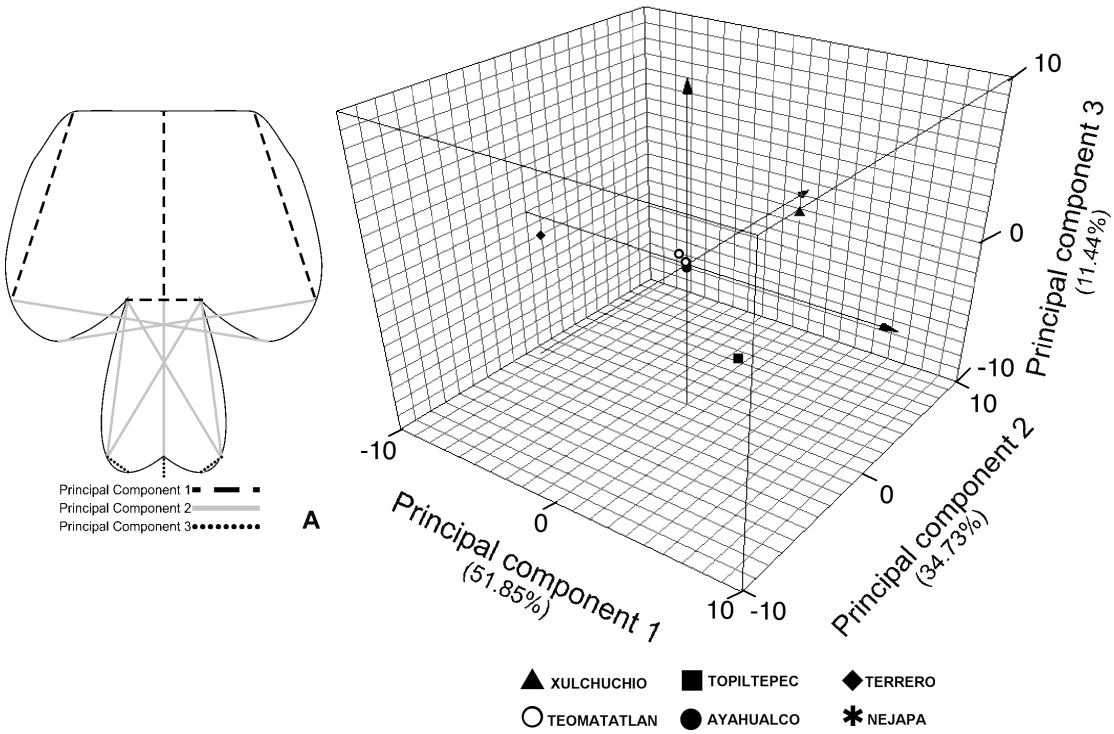

FIGURE 1. Principal Component Análisis Diagram for the lip variation of *L. anceps* subsp. *dawsonii* f. *chilapensis*. A) lip outline showing three principal componentes used in analysis of lip that explain the 98% of the variation.

aesthetic scope in which is inserted the care of the calaveritas. Later when one of its daughters get marriage, the mother inherits to her daughter a cutting of "calaveritas" that will take to its new house and with that will be able to continue with her familiar customs.

The other way of transmission, from mother-in-law to daughter-in-law, is carried out when a woman gets marriage and is gotten to live to mother-in-law´s house. In this situation the wife (daughter-in-law) learns the traditions, values and practices that are made in the backyard or *solar*, from their mother-in-law and when she dies, she will be in charge of the backyard or *solar*, the calaveritas and the continue of the traditions.

It is convenient to indicate that in some cases both situations appear where a woman inherits by the two routes the knowledge and the calaveritas. In opinion of Chilapa women, the knowledge transmission from mother to daughter has greater probability of finalize, since the case of transmission of mother-in-law to daughter-in-law, not always occurs or wether it occurs, is in bad terms because in many cases, the daughters-in-law come from other villages, communities or other states from the country with different customs and practices. On the other hand it is perceived into the community that the current gender role changes, that is to say, the distance of the young woman from the rural life and its incorporation to the labor and urban life, represents a threat for the future transmission of the practices that have allowed the existence of the calaveritas.

Another element recognized as part of the traditional use system, is the valuation factor exists around the calaverita. Into the community predominates the use value or self-comsumption as the main valuation form, which is considered or assigned by the user of the goods from the satisfaction it provides for some of its necessities. For the calaverita, it was verified that this one satisfies spiritual and ornamental aspects that reside in the necessity to honor, to remember and to celebrate the faithfuls deads through the festivity of the day of deads, fundamental activity in the Mexican idiosyncrasy that has worked as incentive in the conservation of the *chilapensis* form.

Finally within the *chilapensis* form specimens lodged in the backyards of Chilapa Guerrero, it was found morphological lip variation. The Principal Component Analysis showed that the first three Principal Components (PC) explained 98% of the variation. The first PC explained 52% and corresponded mainly to the variables related with the size of the mid lobe (long and wide) and to the lateral lobes width. The second PC explained 34% and it´s basically defined by the height of the lateral lobes and the wide one of the base of the mid lobe, whereas the third CP explained 12% of the variation, represented by the height of the apical lobes of the mid lobe. The PCA shows the distribution of four groups within the form *chilapensis* (fig 1). The first group integrated by specimens from the localities of Nejapa, Ayahualco and Teomatatlán, includes plants with quadrangular mid lobe form, with similar wide and length dimensions, in its apical part barely presents 2 formed lobes. The lateral lobes had the highest means values with respect to the rest of the units. The second group is represented by specimens from Topiltepec, it includes plants with piramidal mid lobe, long, thin in the base and wide towards the apex with 2 apicales lobes barely formed. The third group conformed by specimens from Xulchuchio, characterized itself to display a piramidal mid lobe, long, wide in base and towards the apex with 2 strongly pronouncing apicales lobes. And the fourth group represented by specimens from the locality of El Terrero, which was characterized to display a rectangular form mid lobe, narrow and short, in its apical part presents a single lobe.

Under the previous context, it concludes that in the region of Chilapa Guerrero is conserved more than one phenotype of the *chilapensis* form. In the region it is preserved not only one of the more showiest, elegant and attractive forms of the genus *Laelia* in Mexico, but it is also protected the knowledge, traditions and values that gives sustenance to the traditional forms of genetic resources possession. The traditional use of *Laelia* has allowed the conservation of the specie and its variation.

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LITERATURE CITED

Catling, P.M. 1990. *Malaxis salazarii*, a new species from Mexico and northern Mesoamerica. Orquidea (Méx.) 12(1):93-104.

De Garay, G. 1994. La Historia con micrófono. Instituto Mora. México.

Davis, S.D., V.H. Heywood & A.C. Hamilton (eds.) 1994. Centers of Plant Diversity. A Guide and Strategy for their Conservation. Vol. 3. The Americas. World Wide Fund For Nature (WWF) and the World Conservation Union (IUCN). Publications Union Cambridge, UK. 562 p.

Halbinger, F. & M.A. Soto. 1997. Laelias of México. Orquídea (Méx.) Vol. 15. Asociación Mexicana de Orquideologia, A.C. México D. F. México.

Miranda, T. J. 2005. Conocimiento Campesino en el Manejo de la Diversidad de Haba (Vicica faba) en los Llanos de Serdán, Puebla. Tesis de Maestría, Colegio de Postgraduados Campus Puebla. México. pp.144

SAS Institute. 1995 SAS/STAT User's guide, version 6, fiveth edition, volume 1 and 2. SAS Institute Inc. Cary, N. C.

Toledo, V. 1991. El Juego de la Supervivencia: Un Manual para la investigación etnoecológica en Latinoamérica. Consorcio Latinoamericano de Agroecología y desarrollo (CLADES), Santiago de Chile, Berkeley, USA. pp. 75.

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ESTABLISHING A GLOBAL NETWORK OF ORCHID SEED BANKS

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Introduction

Although it would appear that very few orchid species have, as yet, become extinct in the wild, such are the threats to many orchid populations, this situation appears unlikely to continue (Koopowitz, 2001). Just as significant as the loss of individual species is the erosion of genetic diversity within those species. As numbers decline the size of the gene pool reduces, and the rarer alleles begin to disappear. Thus, not only is it important to preserve individual representative specimens, but also the greater genetic variation contained within populations. Seed banks can provide a means of preserving this genetic diversity in a minimum space and at a minimum cost (Linington & Pritchard, 2001). They also have the added benefit of encouraging the raising of orchids from seed, thereby reducing the pressure on wild populations from unscrupulous collectors.

The idea of setting up an orchid seed banking network is not new. It was proposed in 1984 at the World Orchid Conference in Miami (Greatwood, 1984). Despite Lewis Knudson's findings in the 1950's that dry seeds of at least some orchid species could be stored for at least 20 years at refrigerator temperatures (Knudson, 1954), the view nevertheless persisted in some circles at that time that orchid seeds were short-lived. Detailed research over the past twenty years has, however, demonstrated that orchid seeds are no more short-lived than seeds of some other plant families (Pritchard *et al.* 1999). As far as we can tell, the vast majority should remain viable for decades (Seaton & Pritchard, 2003) at the very least when stored dry and at low temperatures. They can therefore be descibed as being, broadly speaking, orthodox. Orthodox seed is seed that can be dried to low moisture contents, and therefore maintained in storage at below-freezing temperatures. There are a few minor differences in the behaviour of orchids to typical orthodox seeds at some sub-zero temperatures (around -30 to -50 C) where, contrary to expectations, longevity is reduced (Pritchard *et al.* 1999). These temperatures are, however, easily avoided, and dry orchid seed can be stored successfully at either 5 C, the temperature of a domestic refrigerator, or -20 C, the temperature of a domestic deep freeze (Seaton & Pritchard, 2003).

Seed Collection and Seed Qualit

Orchid seeds are covered both by the environmental laws of the host countries, as well as by international regulations. Before any seed is collected, permission must be obtained from the relevant authorities. Likewise, seed from cultivated plants must be derived from legally collected plants.

Accurate identification of the parent plant is essential. If the seed bank is attached to a botanical garden a flower can be preserved in spirit, and deposited in the garden's herbarium. An alternative solution would be to submit a good quality photograph of a flower, and perhaps one of the whole plant in flower too. Happily digital photography and scanners make it relatively easy to include such information in a database.

Once permission has been obtained, the next step is the collection of a representative sample of good quality seed. Put simply, you could not reasonably expect poor quality seed to maintain high levels of viability for as long as good quality seed. Quality depends upon a number of variables. These include the age of the flower (and therefore, most likely, of the pollen) at the time of pollination, timing of harvest, parentage, and environmental conditions during maturation of the seed capsule.

In the case of greenhouse-grown plants, the tempta-

tion is to enjoy the flowers for as long as possible before pollinating them. However, the older the flower the older the pollen, and old pollen is itself likely to be less viable. To produce seed of optimum quality, the best advice is probably to pollinate the flowers as soon as they are fully open. Large, strong, well-grown plants, tend to produce more viable seeds than their less robust partners.

Although sowing of immature embryos (so-called 'green pod' techniques) has advantages in terms of reducing the time a capsule is carried by the parent plant, avoiding problems of surface-sterilization of seeds during the sowing procedure and, in the case of some 'hardy' species, circumventing dormancy mechanisms, the likelihood is that such seed cannot be stored as successfully as mature seed. As seeds mature within the capsule they gradually acquire the ability to survive drying, particularly towards the end of the maturation process. Drying is a key component of successful orchid seed storage. Seed is therefore best harvested at, or just prior to, splitting of the seed capsule. This implies a prior knowledge of the phenology of the species in question, and frequent observation as the capsule matures. In order to find the plant once more when out of flower it may be necessary to used coloured tags, or a metal detector if metal tags are employed. Seed should be collected in paper envelopes.

If outcrossing typically leads to more vigorous offspring, the converse is also generally true – selfing, or repeated inbreeding leads to less vigorous offspring. Thus there is strong evidence of inbreeding depression in *Restrepia*, both in terms of a reduction in the percentage of viable seeds produced and the a reduction in vigour of the seedlings (Millner *et al.* 2007 in progress).

Collection of seed samples in dry environments is likely to be a much easier task than that in the humid tropics, where contamination by fungi and bacteria may be a serious problem. Indeed, a more practical option may be to raise plants specifically for seed collection in living collections with their advantages of enabling controlled pollinations and the ability to monitor seed capsule development.

Once harvested, seeds should be examined to check their viability. Ideally a sample can be examined under low magnification using a microscope, but a x10 hand lens will often be sufficient for at least a cursory examination. Potentially viable seed can be recognised by the presence of a plump embryo (Seaton & Ramsay, 2005). Although this is, in itself, no guarantee that the seed will germinate; it may require a special medium or a compatible symbiotic fungus or, in some instances, a dormancy-breaking procedure; the presence of such an embryo in freshly harvested seeds generally suggests the seed is viable. In addition to the obviously full embryos, some seed coats will house somewhat reduced embryos. Others will contain no embryos at all.

Drying Seed to a Suitable Moisture Content

The benefits of reducing seed moisture content are, if anything, greater than those of reducing seed storage temperature. At one extreme, moist seed will support the growth of fungal and bacterial spores, which will multiply rapidly and kill the embryo; infected seed soon become almost impossible to sterilize and to sow in a sterile flask without contamination. At the other end of the spectrum, life processes depend on the presence of moisture. Thus, reducing seed moisture contents to the extreme can dramatically shorten life-spans.

The aim is to obtain a seed moisture content as close to the optimum as is practical (neither too high, nor not too low). If placed in a humid atmosphere, seed will gradually absorb moisture. Likewise, in a dry atmosphere seed will lose water to the air. In either case, over a period of time, the seed and the atmosphere will reach an equilibrium; at which point moisture is neither lost nor gained. The seed will have achieved its equilibrium moisture content.

Where basic laboratory facilities are available a saturated solution of calcium chloride is recommended (lithium chloride might be even better, but there may be problems with availability). A saturated solution of calcium chloride gives a relative humidity around 30 % at 20 C, and seed moisture contents of 4 to 6%. The saturated solution should occupy at least one quarter of the volume of the desiccator (Seaton & Pritchard, 2003). As long as some undissolved salt remains in the solution, the relative humidity will remain constant whilst the container remains at that temperature.

An alternative desiccant

For the amateur or hobbyist, who may have difficulty accessing calcium chloride, dried rice is a suitable alternative. Toasted rice has been used as a desiccant for a wide range of both temperate and tropical seeds (Sadik & White, 1982; Akromah & Bennett-Lartey, 1996). Any supermarket brand will do. Simply spread the rice as a thin layer (no more than one or two grains thick) in the bottom of a baking tray, and dry in the oven at around 100 C or slightly higher overnight.

It is important to remember that the rice will require regular regeneration as, with repeated use, it will itself gradually become increasingly moist. The drying capacities (i.e. how much moisture it is capable of absorbing) of rice is also generally unknown, so you should use plenty of it, filling the desiccator at least three quarters full of dried rice (Seaton & Ramsay, 2005). Dried rice may also be considered the ideal desiccant for use in the field. Where there is a long time interval between harvest and reaching the seed storage facilities, the freshly-harvested seed can be placed in a suitable container in a small desiccator containing dried rice, and then dried to a more optimal seed moisture content on returning to the laboratory.

A word about Silica Gel

As with dried rice, unless it is regenerated each time it is used, silica gel slowly absorbs moisture from the atmosphere, its water absorbing capacity gradually declines, and it produces a different, higher, relative humidity.

A second, and potentially serious, problem with using dry silica gel as a desiccant is that it can produce very low moisture contents indeed: so low that they are potentially damaging to the embryo and actually reduce seed longevity (Seaton & Pritchard, 2003). The use of silica gel as a desiccant, although its use for short-term storage may be acceptable, is not recommended for long term storage.

Storage Temperature

Good quality (high initial germination) dry orchid seed stored in air-tight vessels at a suitable seed moisture content, will maintain its viability at a temperature of 5 C in a domestic refrigerator for many years. Further reducing storage temperature from refrigerator temperature to the temperature of a domestic freezer (around -18 to -20 C) leads to additional increases in seed longevity.

Storage containers

Many people store seeds in paper envelopes. Waxed paper will not take up moisture, and the seed does not stick to it. For long periods of storage (a number of years), however, hermetically sealed tubes are preferable. Glass tubes are preferable to plastic (where seeds tend to adhere to the sides of the tubes). Seeds should be stored in tubes with a volume that provides a minimum of head space above the seeds. Keeping the volume of air in the head space to a minimum when compared to the volume occupied by the seeds means that the seeds will completely dominate the system. This avoids the potential problem of seeds equilibrating to a new and different moisture content within the storage tube during transfer of that tube to the storage room or facility. If the volume of air is kept to a minimum then the moisture within the seed will overwhelm any effect of the moisture content of the surrounding atmosphere.

A potential problem arises with the long-term integrity of any seal. Storage jars, with their combination of a natural rubber seal and a clamp have been demonstrated to be the best available option (Manger *et al.* 2003). At the Millennium Seed Bank Project (MSBP) at Kew the additional precaution is taken of renewing the seals at ten year intervals.

Tubes can be stored within storage jars. After equilibration to a suitable moisture content, seeds are placed within hermetically-sealed tubes. Sachets of blue silica gel can be included to act as an indicator (not as a desiccant) of the performance of seals if any air leaks occur. The aim is to enable us to judge if moist air leaking past the seal into the jar. If it is, the seed should be re-dried and the seal should be replaced.

Every time a tube of seeds is opened the seed will begin to re-equilibrate with the moisture in the atmosphere. There is a choice to be made between re-equilibrating the remainder of the seed lot over an appropriate constant humidity solution before returning to

storage, or storing seed lots in a number of individual tubes which will be opened once only. If the intention is to remove seed samples for sowing at regular intervals, the latter option may be the most appropriate as long as the seed lot is thoroughly mixed at the start, ensuring that as far as possible, each tube is representative of the whole seed lot.

Maintaining a collection

There is more to establishing a seed bank than simply placing the seed in storage and forgetting about it in the safe and secure knowledge that it will still be there for future generations to draw on if and when necessary. Upon arrival at the seed storage facility, the first step is that of cleaning the seed, separating it from the dry tissue of the seed capsule, usually by gentle sieving. Seed germination should then be tested immediately prior to storage, and then at regular (say 10 year) intervals to monitor seed viability. This does two things, it flags up the necessity for regeneration, and contributes to the pool of knowledge.

It is often the simple things that cause the problems. Good record keeping is vital. It is important to label everything at the outset to avoid any possibility of mix-ups. Tubes, packets and jars should all be labelled and dated, and details kept in a note-book and/or on a computer spreadsheet. All the above information needs to be recorded on a suitable database. Species, photograph, provenance, name of donor, date of harvest, date of receipt, percentage germination upon receipt, medium used to germinate the seed - all need to be recorded for future access. None of this can happen without secure, long-term funding for seed storage facilities.

Two types of collection are generally recognised. The first, an active collection, where accessions are withdrawn at regular intervals. A number of short-term seed banks already exist in the world of the hobbyist grower and, because the seed is stored for a few years at the most, these are active seed banks. Such seed banks are suitable for supplying seed for botanical gardens, researchers, hobbyist and commercial growers

In a long-term account, seed is maintained untouched in storage for many years as a reserve, with material only removed if and when there is a need to regenerate plant material for the seed bank. A seed collection should be viewed as a living entity. However long-lived, plants in living collections do have finite life-spans and need to be replaced at regular intervals.

Seed banks can be viewed in the same way as conventional money banks - there will be withdrawals as well as deposits. When an accession is withdrawn we ought to be able to say two things about the seed lot. First, we should be able to say that the seed is viable. There is no point in sending out dead seed, and indeed there may be little point if the viability is very low. Second, we should say what medium the seed can be germinated on. After all, different media will give different percentage germinations. All media are not equally suitable for all species.

What should we store?

Much as we would like to store everything, it would seem wise to establish a list of priorities. Red Lists would appear to be the ideal tool for assessing the conservation status of species, unfortunately there are currently very few orchid species on the 2004 Global Red List. The Conference of the Parties to the CBD adopted the the Global Strategy for Plant Conservation (GSPC) at its meeting in The Hague in 2002. The ultimate and long-term objective of the GSPC is to halt the current and continuing loss of plant diversity through 16 outcome-orientated global targets for 2010. Target 8 is of particular relevance to *ex situ* plant conservation: 60 per cent of threatened plant species in accessible *ex situ* collections, preferably in the country of origin, and 10 per cent of them included in recovery and restoration programmes. If we are to have any chance of meeting the targets we need to begin storing orchid seed immediately. We simply cannot afford to wait until the desired Red Lists are completed.

Conclusion

We are engaged in a race against time. If we don't act quickly, much of the world's diversity, including orchids, will not be available for future generations to enjoy. The setting up of a global network of orchid seed banks is a cost-effective way of meeting this challenge.

LITERATURE CITED

Akromah, R. & S.O. Bennett-Lartey. 1996. Seed drying over toasted rice. Tropic. Sc. 36:32-33.

Greatwoood, J. 1984. Extract from the proceedings of the International Orchid Commission, March, 1984. Amer. Orch. Soc. Bull. 53: 737.

Knudson, L. 1954. Storage and viability of orchid seed. Amer. Orch. Soc. Bull. 22: 260-260.

Koopowitz, H. 2001. Orchid and their Conservation. B.T. Batsford Ltd., London.

Linington, S.H. & H.W. Pritchard. 2001. Gene Banks. Pp.165-181 *in*: S.A. Levin (editor-in-chief). Encyclopedia of Biodiversity. Vol. 3. Academic Press, New York.

Manger, K.R., J. Adams & R.J. Probert. 2003. Selecting Seed Containers for the Millennium Seed Bank Project: a technical review and survey. Pp. 637-652 *in*: R.D.

Millner, H.J., T.C. Baldwin & P.T. Seaton. 2007. A botanical review of the genus Restrepia (Orchidaceae) and its conservation.

Pritchard, H.W., A.L.C. Poynter & P.T. Seaton. 1999. Interspecific variation in orchid seed longevity in relation to ultra-dry storage and cryopreservation. Lindleyana. 14: 92-101.

Sadik, S. & J.W. White. 1982. True potato seed drying over rice. Potato Res. 25: 269-272.

Seaton, P.T. & H.W. Pritchard. 2002. Recent developments in orchid seed banking. Pages 390-396 in J. Clark, W.M. Elliot, G. Tingley & J. Biro, editors. Proceedings of the 16th World Orchid Conference April 1999, Vancouver. The Vancouver Orchid Society. Richmond BC. Canada.

Seaton, P.T. & H.W. Pritchard. 2003. Orchid germplasm collection, storage and exchange. Pp, 227-258 *in*: K.W. Dixon, P. Cribb, S. Kell & R. Barrett (eds.). Orchid Conservation. Natural History Publications (Borneo), Kota Kinabalu, Sabah.

Seaton, P.T. & M.M. Ramsay. 2005. Growing Orchids from Seed. Royal Botanic Gardens, Kew, UK.

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PRODUCTION OF *CYPRIPEDIUM MONTANUM* SEEDLINGS FOR COMMERCIAL VALUE AND REINTRODUCTION INTO RESTORATION PROJECTS: PHASE II

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The mountain lady's slipper (*Cypripedium montanum* Douglas ex Lindl. is a large and charismatic perennial orchid once widely distributed in montane regions of the northwestern United States. This native North American orchid is a member of a genus that has been highly prized by collectors worldwide. *Cypripedium* is now among the most endangered plant genera in the world. As evidence of threats to its survival, a decade ago

Cypripedium was the sole plant genus the World Wildlife Fund placed on its "10 most wanted" list. Also, the accumulative evidence for progressive global warming is a threat to this genus and other temperate terrestrial orchids worldwide. Its survival will continue to be jeopardized unless measures are taken to ensure its persistence. Until recently, mountain lady's slipper seeds were very difficult to germinate in the laboratory.

Through efforts at Kelsey Creek Laboratories (KCL) in 2004, a method was developed to germinate immature seeds at a high frequency and resulting protocorms are now being routinely cultured in the laboratory (Phase I USDA-Small Business Innovative Research Grant # 2004-33610-14304). KCL is collaborating with Andy Huber at GROWISER (Grande Ronde Overlook Wildflower Institute Serving Ecological Restoration) preserve and Nan Vance and Daniel l. Luoma at Oregon State University in Corvallis to better understand this orchid's growth patterns in the laboratory and field, interaction with mycorrhizae, and exploring other methods of culturing and preserving this species, such as cloning, cryogenics, and synthetic seed production.

The GROWISER preserve has a few thousand C. montanum plants that are being tracked from year to year for plant growth, flower and seed production. In addition, the preserve is being used to measure results of restoration capabilities of laboratory-raised seedlings.

Kelsey Creek Laboratories is presently comparing agar-based and liquid-based seed germination of *C. montanum*, and also exploring the feasibility of cloning this species for commercial value. Preliminary results of these studies and others will be included in the poster presentation. This Phase II research is supported by United States Department of Agriculture-Small Business Innovative Research Grant 2005-03191.

Roger H. Smith,, PhD, and **Jane A. Smith** PhD, are co-owners of Kelsey Creek Laboratories, a custom orchid laboratory serving orchid nurseries and hobbyists through seed germination and mericloning. They have been in business for eleven years. In addition, they have a research project on *Cypripedium montanum*, a mountain lady's slipper, a threatened species in the Pacific Northwest of USA and Canada. The research has been supported by two consecutive grants from the US Department of Agriculture-Small Business Innovative Research (USDA-SBIR) program. Kelsey Creek Laboratories is evolving toward focusing more on threatened and endangered terrestrial orchids of temperate climates.

Scott Liebler, BS, ia a technician at Kelsey Creek Laboratories and focuses primarily on mycorrhizae and terrestrial orchid seed and seedling interactions.

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EXPERIMENTAL REINTRODUCTION OF THE THREATENED TERRESTRIAL ORCHID *DIURIS FRAGRANTISSIMA*

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Introduction

Diuris fragrantissima D. L. Jones et M. A. Clem. is a perennial terrestrial orchid endemic to the state of Victoria, Australia. The species is listed as Critically Endangered in Victoria under the criteria of the International Union for the Conservation of Nature and Natural Resources (IUCN), having suffered a severe population decline since the 1930s, largely attributable to habitat destruction for agricultural and urban development. A recovery plan has been developed by the Department of Sustainability and the Environment (DSE, Victoria, Australia), which includes reintroduction as an important conservation strategy. There is, however, limited research on terrestrial orchid reintroductions, and many of the previous reintroductions reported have been unsuccessful, including three reintroductions of *D. fragrantissima* in the 1980s, in which the majority of plants survived less than 6 months (LaTrobe University, 1982, 1983) (D. Tonkinson pers comm.. 2006). The last of the plants is thought to have died out after 1991 (Murphy *et al*. 2006), with the exception of an anecdotal report of a flowering plant in 2000 (Knight *et al*. 2003). The reasons that these trials were unsuccessful are unknown, largely because of a lack of published data. While *in situ* management should be the first priority for species conservation (Ramsay and Dixon 2003, Vallee *et al*. 2004), the limited success of management action in the preservation of *D. fragrantissima* at the remnant site, and the continual destruction of known secure habitat, has resulted in reintroduction as a major conservation option. An adaptive management approach involving experimental trials will aid understanding of the system and allow management practices to adapt to the requirements of the orchid.

A comparable level of genetic diversity exists between *in situ* and *ex situ Diuris fragrantissima* and its more common relatives (Smith *et al*. 2006), indicating that the *ex situ* collection contains sufficient diversity for retaining evolutionary potential to adapt to long-term environmental change, a requirement for the long-term persistence of reintroduced populations (Vallee *et al.* 2004). In addition, suitable mycorrhizal fungi have been isolated and cultured for use in reintroductions (Smith 2006), which is one of the major steps in the recovery plan (Murphy *et al*. 2006). The inoculation of habitat soils with suitable mycorrhizal fungi is suggested as a prerequisite to ensure that reintroduced orchids survive the transition from *ex situ* to *in situ* (Batty *et al*. 2001). Furthermore, the reliance of terrestrial orchid seed germination on infection with a mycorrhizal fungus (Batty *et al.* 2001) means that fungal presence in the soil is required for further *in situ* seed germination, for development of self-sustaining populations. After reintroduction of orchids and their associated fungi, the persistence of the mycorrhizal fungi must be ensured through the management of site conditions conducive to the survival of fungi and maintenance of the mycorrhizal relationship (Ramsay and Dixon 2003). Soil aeration via tilling may encourage persistence of the mycorrhizal fungi since the soil at the reintroduction site has become compacted in the last 100 years (C. Knight pers. comm. 2006), probably the result of agricultural practices. The addition of fungus

to habitat soil is the most effective way of providing sufficient mycorrhizal inocolum support in orchid translocations (Ramsay and Dixon 2003). Orchidaceous mycorrhizal fungi are not known to readily produce fruiting bodies (Rasmussen 2002) and the hyphae are too fine to observe in the rhizosphere. Therefore, the persistence of orchid mycorrhizal fungi after reintroduction to new sites has not previously been investigated. The loss of associated fungi after reintroductions of symbiotic *D. fragrantissima,* or the inability of asymbiotically germinated plants to form mycorrhizal associations after reintroduction *in situ,* may be one reason for the lack of survival of previously reintroduced plants.

This study investigated the effects of soil aeration and inoculation with mycorrhizal fungus on reintroduced plant survival and persistence, when planted at different times (spring, summer, autumn) and at different stages of the plant life cycle. The persistence of the mycorrhizal relationship one year after reintroduction was investigated to assess the potential long-term success of this reintroduction. Results of this research were expected to aid establishment of a reintroduced population and thereby fulfil one of the major goals of the *Diuris fragrantissima* recovery plan (Murphy *et al.* 2006).

Methods

Experimental reintroductions, involving source *ex situ Diuris fragrantissima,* compared planting as actively growing symbiotic seedlings in spring and autumn and as dormant tubers in summer. The spring and autumn reintroduced plants were incorporated into randomised treatments involving soil aeration and addition of support inoculum, grown on sterile millet seed. Treatment combinations were also incorporated into the experimental design. Insufficient numbers of dormant tubers were available for incorporation into all trials in the experimental reintroduction. Rather, a combination of soil aeration and support inoculum was applied to all reintroduced dormant tubers. Reintroductions were aimed for plants at 2-3 years of age, when tubers were considered to contain sufficient food storage for survival from nursery to field conditions. Site selection was based on soil properties, the previous range of *D. fragrantissima,* and conservation status, security and management of the land.

Source *ex situ* plants were found to be associated with fungi that were genetically similar to *Tulasnella calospora* and to fungi isolated from remnant *in situ* plants, and had the ability to initiate germination of host plants (Smith 2006). The most active fungal isolate obtained from *ex situ* plants, in terms of seed germination initiation, was used as the source inoculum for this research. Sterile millet seed was inoculated with this isolate for transfer to soil. Control germination trials were conducted prior to undertaking the reintroduction to ensure the viability of the fungi. All potting mix was removed from the plants prior to incorporation into 1 m^2 treatment plots, five to each plot. All tuber weights were recorded prior to planting. Monthly monitoring of leaf length, width, stem height, flower and bud number and plant health was conducted between planting (September 2004, December 2004, April 2005) and April 2006.

Analysis of variance (ANOVA) was conducted on the resultant aggregated dataset using the General Linear Model: Univariate option in SPSS 14.0 (SPSS inc. 2005). Graphs were plotted using Microsoft Excel. It was hypothesised that tuber weight would be positively correlated with all variables, so where the correlation was significant, tuber weight was incorporated into the analysis as a covariate to account for any influence on results. Correlation between plants that flowered in 2004 and 2005, and those that re-emerged in seasons following flowering were examined using cross tabulations and bivariate correlations. Significance was noted when $p < 0.05$ for all analyses. One year after reintroduction, fungi were isolated from 20 randomly collected plants, cultured and genetically identified by direct sequencing of the ITS region. Ten of the sampled plants were reintroduced with additional inoculum and ten were reintroduced with only the fungi present in the roots at the time of planting. Seed baiting was also conducted (following Rasmussen and Whigham 1993) to determine whether fungi persisted in soil one year after reintroduction in association with host orchids and alone.

Results and Discussion

Planting as actively growing seedlings in spring and autumn was far more successful than as dor-

mant tubers in summer, with 69% (spring) and 46% (autumn) plants persisting after one year, compared to only 16% of plants reintroduced as dormant tubers in summer. Tuber size was positively correlated with plant size and health, confirming anecdotal reports that plants with larger tubers were more successful in reintroductions. A combination of support inoculum and soil aeration significantly improved plant growth and survival in the spring reintroduction but had no significant effect on plants reintroduced in autumn. Fungal inoculum support alone did not improve plant growth or survival in either planting time. Source plants for reintroduction were propagated asymbiotically *in vitro* and became associated with fungi in potting media. The fungi present in plant roots at the time of reintroduction were sufficient to support the transition from nursery to field. Therefore, the persistence of actively growing symbiotic seedlings in reintroductions shows that *Diuris fragrantissima* can be inoculated post-germination for successful reintroduction. Optimum monitoring dates for reintroduced *D. fragrantissima* were determined to be July, when most plants were emergent, and late October to early November, when stem height and flowering were at their greatest.

Fungi associated with *Diuris fragrantissima* plants at the time of reintroduction were re-isolated and identified one year after planting. Nine of ten sampled plants that were reintroduced into plots inoculated with fungi originally grown on sterile millet were found to have become associated with the support inoculum. Therefore, mycorrhizal association of symbiotic reintroduced plants was maintained for at least a year, and plants were able to form new associations with fungi post-planting. Although reintroduced plants became associated with fungi inoculated into recipient site soil, the support inoculum did not appear to increase plant growth or survival, unless combined with soil aeration for plants reintroduced in spring. The fungi present in the underground organs of *D. fragrantissima* was sufficient to support growth and flowering in two seasons.

This study was successful in the development of a reintroduction program for *Diuris fragrantissima,* and highlights the information necessary to implement reintroductions as effective conservation strategies. Extended monitoring of the reintroduced population is required to determine the long term success, including optimising requirements for recruitment of seedlings *in situ,* by means of pollination and seed germination.

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LITERATURE CITED

Batty A.L., K.W. Dixon, M.C Brundrett & K. Sivasithamparam. 2001. Long-term storage of mycorrhizal fungi and seed as a tool for the conservation of endangered Western Australian terrestrial orchids. Aust. J. Bot. 49, 619-628.

Knight, C., E.A. James, S. Akiyama & L. Pryde. 2003. Molecular biology and pollination as a tool to enhance the long-term viability of the Sunshine Diuris (*Diuris fragrantissima* D. L. Jones et M. A. Clem). Final report to the Hermon Slade Foundation.

Murphy A.H., C. Knight, G. Backhouse & A. Webster. 2006. Recovery plan for the Sunshine Diuris, *Diuris fragrantissima* (Orchidaceae: Diuridinae) 2002-2007. Department of Sustainability and Environment, Melbourne, Victoria, Australia.

Ramsay M.M., & K.W. Dixon. 2003. Propagation science, recovery and translocation of terrestrial orchids. Pp. 259-288 *in* Dixon, K.W., S.P. Kell, R.L. Barrett, R.L. & P.J. Cribb (eds.). Orchid conservation. Natural History Publications (Borneo): Kota Kinabalu, Sabah

Rasmussen H.N. 2002. Recent developments in the study of orchid mycorrhiza. Plant and Soil 244, 149-163.

Rasmussen H.N. & D.F. Whigham. 1993. Seed ecology of dust seeds *in situ*: A new study technique and its application in terrestrial orchids. Am. J. Bot. 80, 1374-1378.

Smith, Z.F. 2006. Developing a reintroduction program for the threatened terrestrial orchid *Diuris fragrantissima.* Ph.D. thesis, The University of Melbourne, Victoria, Australia.

Smith, Z.F., E.A. James & C.B. McLean (In press). Investigation of the phylogenetic relationships between *Diuris fragrantissima* and its closest relatives using AFLPs. The Environmentalist.

SPSS Inc. 2005. SPSS version 14.0 for Windows. © SPSS Inc. Headquarters, Chicago, USA.

Vallee L., T. Hogbin, L. Monks, B. Makinson, M. Matthes & M. Rossetto. 2004. Guidelines for the translocation of threatened plants in Australia. Australian Network for Plant Conservation: Canberra, Australia.

Zoë Smith recently obtained her Ph.D. in resource management at the University of Melbourne, Australia. Her research focussed on the taxonomic status, genetic diversity, fungal ecology and systematic relationships, and reintroduction of *Diuris fragrantissima.* During her studies she was employed as an orchid researcher at the Royal Botanic Gardens Melbourne, and also held a short internship at the Royal Botanic Gardens, Kew, UK, where she conducted research on species extinction modelling. Zoë is currently employed as an environmental consultant and is looking for a post doctoral position.

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FINDING A MYCORRHIZAL FUNGUS FOR REINTRODUCTIONS OF THE THREATENED TERRESTRIAL ORCHID *DIURIS FRAGRANTISSIMA*

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Australian terrestrial orchids rely on associations with suitable mycorrhizal fungi for *in situ* seed germination and establishment, an important prerequisite for self sustaining populations. Finding an appropriate mycorrhizal fungus is therefore imperative to successful reintroductions. Reintroductions have been planned to conserve the terrestrial orchid *Diuris fragrantissima*, which is Critically Endangered in Victoria, Australia, having been reduced to less than 25 plants at a single site.

This study investigated the presence of a suitable mycorrhizal partner for *Diuris fragrantissima in situ, ex situ* and from closely related species, for use in reintroductions. Six hundred seed baits were placed at three original sites of *D. fragrantissima* but did not recover a single germinant. Twenty-two fungi isolated from *D. punctata*, *D. dendrobioides* and *D. chryseopsis*, and ten fungi isolated from *D. fragrantissima* in *ex situ* collection were used in germination trials with seed of *D. fragrantissima*. Three isolates initiated germination, including fungi isolated from 'asymbiotic' *ex situ D. fragrantissima*. Germination rates were always below 30%. Fungal isolates were identified by direct sequencing of the nuclear internal transcribed spacer and large subunit regions of DNA. All isolates were closely related to *Tulasnella calospora*.

Evolutionary relationships between fungi and their orchid hosts across Victoria are discussed.

Zoë Smith recently obtained her Ph.D. in resource management at the University of Melbourne, Australia. Her research focussed on the taxonomic status, genetic diversity, fungal ecology and systematic relationships, and reintroduction of *Diuris fragrantissima.* During her studies she was employed as an orchid researcher at the Royal Botanic Gardens Melbourne, and also held a short internship at the Royal Botanic Gardens, Kew, UK, where she conducted research on species extinction modelling. Zoë is currently employed as an environmental consultant and is looking for a post doctoral position.

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ORCHID CONSERVATION IN THE AMERICAS—LESSONS LEARNED IN FLORIDA

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Introduction

Conservation can be a difficult concept to define, let alone apply. For some, plant conservation can simply mean the survey and cataloging of plant diversity within a defined conservation area. Others may define plant conservation as the collection and propagation of germplasm from one, or many, areas within a species' range. Still others may define plant conservation as the act of plant translocation for population reintroduction, establishment, or augmentation. Traditionally, many plant conservation researchers have focused efforts on only one or two of these actions and defined their efforts as effective species-level conservation. These researchers have treated species-level conservation as a set of individual steps that have little, or no, interconnection. Orchid species conservation has traditionally taken on this separated approach. Few of the many traditional orchid conservation efforts have taken into account one integrated picture of the orchid plants, their pollinators and reproduction, their mycobionts, their propagation, and their population genetic diversity.

Florida is home to approximately 120 orchid species and a number of other growth and color forms and varieties (Brown 2005). While nearly all of these species are considered endangered, threatened, or commercially-exploited, few have received any conservation attention and even less have received attention from those interested in integrated conservation. In 2002 the Plant Restoration, Conservation, and Propagation Biotechnology Program at the University of Florida (Gainesville, Florida) established a partnership with the Florida Panther National Wildlife Refuge (Naples, Florida) aimed at conserving the orchids of Florida through the implementation of integrated conservation efforts at the species level. To date, the partnership has initiated research examining various factors of the ecology, biology, and propagation of several native Florida orchids, including *Bletia purpurea* (Lamark) de Candolle, *Cyrtopodium punctatum* (Linnaeus) Lindley, *Dendrophylax lindenii* (Lindley) Bentham *ex* Rolfe, *Eulophia alta* (Linnaeus) Fawcett & Rendle, *Habenaria macroceratitis* Willdenow, *Prosthechea cochleata* (Linnaeus) W.E. Higgins var. *triandra* (Ames) W.E. Higgins, *Spiranthes floridana* (Wherry) Cory *emend*. P.M. Brown, and *S. odorata* (Nuttall) Lindley. Through the use of field observations of plants and pollinators, isolation and characterization of mycobionts, symbiotic and asymbiotic seed germination, and the study of population genetic diversity by amplified fragment length polymorphism (AFLP), the partnership has been able to demonstrate the applicability of integrated conservation methods for the conservation of orchid species in Florida.

The acceptance and application of these methods outside of Florida is now needed to further demonstrate their effectiveness on a global scale. The methods, successes, failures, and tribulations of identifying and addressing integrated orchid conservation needs in Florida will be discussed. Additionally, several case studies will be outlined will be used to elucidate these methods. One example case study on *H. macroceratitis* is presented here.

Case Study

Habenaria macroceratitis.—The following is a brief case study outlining the major areas of research concerning the integrated conservation of *H. macroceratitis* in Florida. At the time of writing, some data

concerning the species' ecology, pollination biology, and population genetic diversity was not fully analyzed and, therefore, not presented in the current paper.

PLANT ECOLOGY. *Habenaria macroceratitis* is a rare sub-tropical terrestrial orchid native to central Florida, Mexico, the West Indies, and Central America (fig. 1). The species typically inhabits moist hardwood hammocks in Florida where the canopy is dominated by *Quercus virginiana* (live oak), *Magnolia grandiflora* (magnolia), *Liquidambar styraciflora* (sweet gum), *Pinus elliottii* (slash pine), *P. palustris* (long-leaf pine), and *Sabal palmetto* (cabbage palm) (S.L. Stewart, unpub. data 2003). The loss of hardwood hammock habitat throughout Florida has prompted interest by researchers and state agencies in the preservation and restoration of these critical habitats (Maehr & Cox 1995, Sprott & Mazzotti 2001). Many native Florida terrestrial orchids, including *H. macroceratitis*, inhabit this threatened habitat (S.L. Stewart, pers. observ. 2003) and unless effective species-level conservation methods are developed, these species are likely to face population decline or extinction. The integrated conservation of *Habenaria macroceratitis*, the long-horned rein orchis, was investigated through an examination of the species' ecology, mycology, propagation science, pollination biology, and population genetic diversity.

Four populations of *H. macroceratitis* were identified in west-central Florida representing various population sizes and conservation statuses. One of the populations was considered large (Hernando County #1, 270 plants) and existed on privately-owned land that was being adaptively managed for the orchid-appropriate habitat. The second population was considered of moderate size (Hernando County #2, 65 plants) and existed on privately-owned land with no management being applied. The final two populations were considered small (Marion County, 16 plants; Sumter County, 10 plants), with the Marion County population existing on managed state-owned land and the Sumter County population existing on privately-owned non-managed land. Data concerning flowering versus vegetative production, plant demographics, and habitat characterization were collected. At the time of writing, these data are currently being analyzed for associations among habitat/environmental factors and whole plant responses.

FIGURE 1. *Habenaria macroceratitis* inflorescence in habitat.

PLANT MYCOLOGY. — Fifteen mycobionts were isolated from the roots of *H. macroceratitis* collected at two of the previously mentioned populations (Hernando and Sumter Counties) following the methods outlined by Zettler (1997) and Stewart and Zettler (2002). Six of the mycobionts were assignable to the anamorphic fungal genus *Epulorhiza* Moore, while the remainder were assignable to the anamorphic

genus *Ceratorhiza* Moore, based on cultural morphology, microscopic examination, and enzyme assays. These mycobionts were subsequently used in a number of studies investigating the *in vitro* symbiotic seed germination of *H. macroceratitis*. These data suggest that *H. macroceratitis* may demonstrate a degree of fungal specificity based upon geographic area in central Florida. Therefore, to insure the conservation of this species through symbiotic seed germination mycobionts from many populations throughout the species' Florida range should be isolated, characterized, and cataloged.

PLANT PROPAGATION. — Of particular interest was the development of a reliable symbiotic seed germination method that accounted for any potential mycobiont specificity based on seed collection and mycobiont collection sites. Current data suggest that symbiotic germination of this species is possible and that this species does demonstrate a degree of mycobiont specificity when seeds and mycobiont collected from the same sites versus geographically distinct sites were co-cultured (Stewart & Kane 2006b). These data suggest that the distribution of *H. macroceratitis* in west-central Florida may be closely tied with the distribution of specific mycobionts and not the species' typical hardwood hammock habitat. This finding has the potential to change state conservation policy concerning the management of hardwood hammock habitats throughout Florida to integrate management strategies for plants, animals, and soil microflora.

Concurrent with the symbiotic studies, the asymbiotic seed germination of *H. macroceratitis* was undertaken in an attempt to better understand the processes of seed germination and seedling development in the species. A simple asymbiotic media screen using six germination media was conducted to determine an optimal asymbiotic germination medium for further downstream studies. These media included Modified Lucke, Murashige & Skoog, Lindemann, Vacin & Went, Malmgren Modified Terrestrial Orchid Medium, and Knudson C. After 16 wks dark incubation, the Malmgren Modified medium supported the highest final percent germination and, therefore, was chosen to support further studies on seed germination and seedling development (Stewart & Kane 2006a). Subsequently, the effect of three photoperiod treatments (0/24, 16/8, 24/0 h L/D) on asymbiotic seed germination were evaluated. Seeds incubated in continual darkness (0/24 h L/D) exhibited the highest final percent germination (91.7%) and the most advanced protocorm developmental stage (Stage 4, fig. 2, Stewart & Kane 2006a). Although seed germination under both 16/8 and 24/0 h L/D treatments was stimulated, percent germination was statistically lower (fig. 2, Stewart & Kane 2006a). These data reinforce the notion that, in general, seeds of terrestrial orchid species are best germinated in continual darkness and that exposure to light may inhibit germination or protocorm development.

To further examine *in vitro* growth and development of *H. macroceratitis*, the effects of three photoperiod treatments (8/16, 12/12, 16/8 h L/D) on *in vitro* seedling development was evaluated. Asymbiotic seedlings grown under a 8/16 h L/D photoperiod produced the highest number of tubers (1.06), the tubers with the greatest fresh and dry mass (42.7 µg and 6.5µg, respectively), and the largest diameter tubers (3.1 mm) as compared to those seedlings grown under the other photoperiod conditions (Stewart & Kane 2006a). Interestingly, leaf number and size (length and width) significantly decreased as photoperiod increased from 8/16 to 16/8 h L/D (Stewart & Kane 2006a). These data suggest that the tuber formation response seen in *in situ* plants of *H. macroceratitis* in response to a decrease in photoperiod is maintained under *in vitro* conditions. This response could be harnessed to produce *in vitro* tubers of the species that may be better suited to translocation to restored habitats than leaved seedlings.

PLANT POLLINATION BIOLOGY. — The pollination biology of *H. macroceratitis* was investigated to determine what role an insect pollinator may play in the long-term conservation of the species. Following the methods outlined by Zettler *et al.* (1996) pollinator observations, nectar volume, and nectar sugar concentrations were conducted on 26-28 August 2004. Given that *H. macroceratitis* has white to cream colored flowers and produced a scent only at night, it was not surprising that a night-flying moth

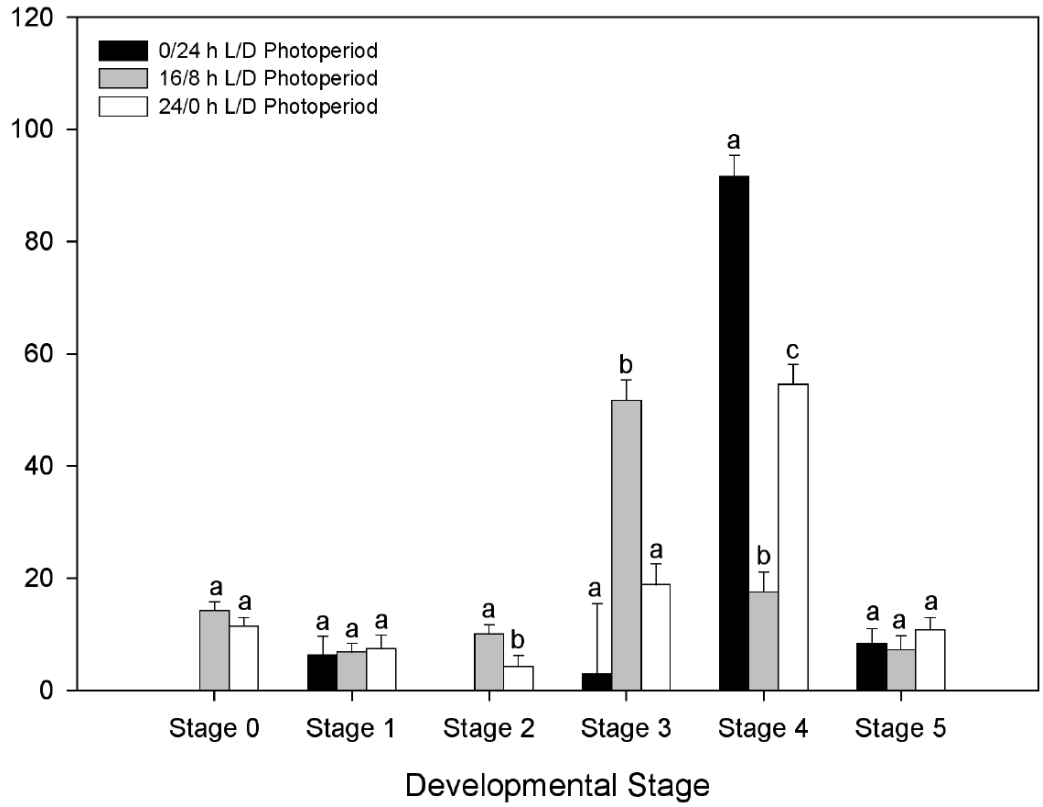

FIGURE 2. Effects of three photoperiod treatments (0/24, 16/8, 24/0 h L/D) on asymbiotic seed germination of *Habenaria macroceratitis* cultured on Malmgren Modified Terrestrial Orchid Medium after 14 weeks. Histobars with same letter are not significantly different within stage (? = 0.05).

(*Cocytius antaeus*, giant sphinx moth) was observed visiting and probing individual flowers. Furthermore, nectar volume (4.25 µl) and sugar concentration (max. 20%) were highest in the early evening, presumably in preparation for optimal attraction timing of *C. antaeus*.

Pollination mechanism experiments were designed to further investigate the breeding system of *H. macroceratitis*. Methods followed those outlined by Wong and Sun (1999), modified with the inclusion of seven experimental pollination mechanism conditions—open pollination (control), agamospermy, spontaneous autogamy, induced autogamy, artificial geitonogamy, artificial xenogamy, and induced xenogamy. Seed capsules were formed in four of the seven treatments—open pollination, induced autogamy, artificial geitonogamy, and artificial xenogamy. *Habenaria macroceratitis* does not appear to be self-fertile, agamospermic, or self-pollinating. Tetrazolium staining revealed high seed viability from three of the four successful pollination treatments, with open pollination being the most viable (91.0%) followed by artificial geitonogamy (86.3%) and induced autogamy (76.8%). The artificial xenogamy treatment resulted in 50.7% viable seed.

Examining the combined pollinator observation and pollination mechanism data, *H. macroceratitis* appears to rely on its night scent and nectar reward to attract nocturnal sphinx moths, such as *C. antaeus*. However, the data demonstrate that these nocturnal pollinators appear to transfer pollen within isolated populations of *H. macroceratitis*. This short distance pollen movement appears to result in highly viable seed within populations, but when pollen from a distant population is brought into an isolated population

a decrease in seed viability results. These findings may indicate some level of historic isolation among *H. macroceratitis* populations in central Florida, and therefore result in a management plan that maintains this degree of population isolation.

PLANT POPULATION GENETIC DIVERSITY. — One of the final steps in the integrated conservation of *H. macroceratitis* is the examination of population genetic diversity within and among populations in central Florida. The AFLP technique (Vos *et al.* 1995), modified by Ranamukhaarachchi *et al.* (2000), will be used to assess the population genetic diversity of *H. macroceratitis*. Characterization of population genetic variability may help to better manage the conservation and restoration of *H. macroceratitis* habitat, as well as improve potential plant translocations of the species.

At the time of writing, a DNA library has been compiled representing 75 and 18 plants, respectively, from the Hernando County #1 and #2 populations, 14 plants from the Marion County population, and 7 plants from the Sumter County population. The AFLP technique is currently being applied to these samples. Genetic diversity in *H. macroceratitis* is expected to be representative of fit populations existing in isolated habitat pockets throughout central Florida. If this hypothesis is demonstrated as true, management for this species should focus on the maintenance of these isolated population and the conservation or restoration of the species' endangered habitat.

Conclusions

Integrated conservation represents a best management practice for the species-level conservation of orchid species. The effectiveness and applicability of this integrated approach has clearly been demonstrated in Florida through the research conducted on *H. macroceratitis* via the partnership between the Plant Restoration, Conservation, and Propagation Biotechnology Program at the University of Florida and the Florida Panther National Wildlife Refuge. In examining multiple levels of species plant conservation—from broad ecology to specific population genetic diversity—a holistic approach to the conservation of *H. macroceratitis* has been developed.

The outline of species-level integrated conservation, as presented here, represents a generalized scheme and should be interpreted as a set of guidelines only. Differences in species and habitats, as well as differences in conservation goals, political and social structure, and funding are all responsible for shaping the integrated approach and expected outcomes. Others interested in orchid conservation on either the species- or landscape-level are encouraged to design and implement this integrated approach.

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LITERATURE CITED

Brown, P.M. 2005. Wild orchids of Florida. University Press of Florida, Gainesville, FL.

Maehr, D.S & J.A. Cox. 1995. Landscape features and panthers in Florida. Conserv Biol 9: 1008-1019.

Ranamukhaarachchi, D., M.E. Kane, C.L. Guy & Q.B. Li. 2000. Modified AFLP technique for rapid genetic characterization in plants. BioTechniques 29: 858-866.

Sprott, P. & F.J. Mazzotti. 2001. Habitat loss, Florida's changing landscape: upland forests. WEC 151, Cooperative Extension Service, Institute of Food and Agricultural Science, University of Florida, Gainesville, FL.

Stewart S.L. & M.E. Kane. 2006a. Asymbiotic seed germination and *in vitro* seedling development of *Habenaria macroceratitis* (Orchidaceae), a rare Florida terrestrial orchid. Plant Cell Tiss Organ Cult 86: 147-158.

Stewart, S.L. & M.E. Kane . 2006b. Symbiotic seed germination of *Habenaria macroceratitis* (Orchidaceae), a rare Florida terrestrial orchid. Plant Cell Tiss Organ Cult 86: 159-167.

Stewart, S.L. & L.W. Zettler . 2002. Symbiotic germination of three semi-aquatic rein orchids (*Habenaria repens*, *H. quinqueseta*, *H. macroceratitis*) from Florida. Aquat Bot 72: 25-35.

Vos, P., R. Hogers, M. Blecker *et al.* 1995. AFLP: a new technique for DNA fingerprinting. Nucl Acids Res 23: 4407-4414.

Wong, K.C. & M. Sun. 1999. Reproductive biology and conservation genetics of *Goodyera procera* (Orchidaceae). Am J Bot 86: 1406-1413.

Zettler, L.W. 1997. Terrestrial orchid conservation by symbiotic seed germination: techniques and perspectives. Selbyana 18: 188-194.

Zettler LW, Ahuja NS, McInnis TM. 1996. Insect pollination of the endangered monkey-face orchid (*Platanthera integrilabia*) in McMinn County, Tennessee—one last glimpse of a once common spectacle. Castanea 61: 14-24.

Scott Stewart, currently a Ph.D. candidate in the Environmental Horticulture Department at the University of Florida, has been researching orchid conservation practices since 1999. Although initially trained working with orchids native to the temperate Midwest United States, he has a more recent interest in the biology and conservation of sub-tropical and tropical terrestrial orchids. Of particular interest to Scott are the genera *Spiranthes*, *Habenaria*, and their segregates.

Michael Kane, Ph.D., Assistant Chair and Professor of Environmental Horticulture, conducts research on the application of plant *in vitro* culture techniques for production of native plants, including orchids, wetland and coastal plants for the purposes of habitat restoration and conservation. His research incorporates the use of genetic markers to characterize plants, *in vitro* culture procedures as well as greenhouse and field studies.

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PROPAGACIÓN *IN VITRO* Y ACLIMATIZACIÓN DE *EUCHILE MARIAE* (AMES) WITHNER (ORCHIDACEAE)

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ABSTRACT. Orchid micropropagation of species *Euchile mariae* was achieved from the *in vitro* culture of protocorms sections, obtained from the germination of seeds. The top and bottom protocorms sections, used as explants, were cultivated in modified MS culture medium, added with different concentrations of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA). The morphogenetic response obtained of both types of explants, was the formation of protocorm like bodies (PLB's), that gave place to the formation of new plantlets. Of two tested explants the biggest formation of PLB's was obtained from the bottom protocorms sections. The acclimatization of the plantlets was successful, with a 100% percent survival. Plant cell tissue culture represents a useful alternative for the study, conservation and massive propagation of this and other endangered species.

PALABRAS CLAVE: micropropagación, orquídeas, *Euchile*, PLB's, aclimatización, conservación *ex situ*

Introducción

México es un país megadiverso, se estima que alberga más del 12% de la biota del planeta (Mittermeier 1988, Toledo 1994), ocupando el cuarto lugar en diversidad vegetal a nivel mundial (Magaña & Villaseñor 2002), resultado de la combinación de distintos factores como su historia geológica, posición geográfica, diversidad de altitudes, climas y orografía que presenta (Ramamoorthy *et al.* 1993). Aunado a estos factores presenta una gran cantidad de endemismos; se estima que entre el 10% y 15% de los géneros y 54.2% de las especies de plantas vasculares de México son endémicas (Magaña & Villaseñor 2002), siendo las Orchidaceae una de las familias de plantas con mayor número de especies endémicas. De las 20, 000 a 30, 000 especies de orquídeas que se reportan a nivel mundial (Dressler 1981, Arditti 1992, Dressler 1993, Espejo *et al.* 2002, Hágsater *et al.* 2005), México cuenta con aproximadamente 1106 especies y subespecies mexicanas descritas, distribuidas en 159 géneros, de las cuales el 40% son endémicas, es decir, aproximadamente 444 especies (Soto 1996). Un dato más reciente sugiere que se distribuyen cerca de 1300 especies, agrupadas en 170 géneros (Soto & Salazar 2004). Desafortunadamente, México presenta graves problemas ambientales y sociales que ponen en peligro parte importante de su biodiversidad (Hágsater *et al.* 2005); de manera que la orquideoflora mexicana se encuentra seriamente amenazada, principalmente por actividades humanas, tales como la deforestación, erosión de suelos, reducción y fragmentación de hábitats y la extracción ilegal de especies (UICN /SSC Orchid Specialist Group 1996). Esto demuestra que es urgente e indispensable establecer estrategias de conservación para esta familia de plantas.

Euchile mariae (Ames) Withner, es una orquídea epífita y endémica de México que se distribuye en los estados de Tamaulipas, San Luis Potosí, Hidalgo, Guanajuato, Querétaro, Puebla y Veracruz. Habita en bosques de encino, pino-encino y liquidámbar, a una altitud de 800 a 1350 metros sobre el nivel del mar (Soto 2002). Se encuentran catalogada por la Norma Oficial Mexicana (NOM-059-ECOL-2001) como una especie amenazada (SEMARNAT 2002), esto debido principalmente a la destrucción de su hábitat y al saqueo y comercio ilegal de plantas silvestres. Así mismo, al igual que muchas otras especies de

orquídeas es una especie de lento crecimiento y largo ciclo de vida por lo que existe escaso reclutamiento de nuevos individuos en condiciones naturales (Ávila & Oyama 2002). En este sentido, el Cultivo de Tejidos Vegetales proporciona una excelente herramienta para el estudio, propagación y conservación de estas especies. El presente estudio describe un protocolo para la micropropagación de *Euchile mariae* mediante el cultivo de secciones de protocormos y el efecto de los reguladores de crecimiento ANA y BA para inducir una respuesta morfogenética a partir de estos explantes.

Materiales y Métodos

Se utilizó una cápsula de *Euchile mariae* antes de su dehiscencia, ésta se colocó en una solución de hipoclorito de sodio al 30% (v/v), durante 30 minutos en agitación constante, seguido de tres enjuagues con agua destilada estéril dentro de la campana de flujo laminar, en donde se colocó sobre una caja petri estéril y se disectó con ayuda de pinzas y bisturí. Con una espátula se tomaron las semillas de la cápsula y se sembraron en frascos de 125 ml de capacidad conteniendo 25 ml de medio MS modificado (Murashige & Skoog 1962).

El pH del medio de cultivo se ajustó a 5.5 con soluciones de NaOH y de HCl a 0.1, 0.5 y 1N, antes de agregar 4 g/l de gelrite. Los frascos con el medio de cultivo fueron esterilizados en un autoclave a 121° C y 1.5 kg/cm^2 durante 15 minutos. Todos los cultivos fueron incubados a 25 $\pm$ 1° C , con un fotoperiodo de 16 h luz y 8 h de obscuridad y 50 µMol/m/s de intensidad luminosa.

Los explantes utilizados para iniciar los ensayos de inducción fueron mitades de protocormos cuando éstos tenían un tamaño promedio de 2-5 mm de longitud y presentaban el primer primordio foliar. En condiciones de asepsia, los protocormos se colocaron sobre una caja Petri estéril, en donde fueron disectados transversalmente con ayuda de pinzas de disección y bisturí, obteniéndose así secciones apicales y basales de protocormos. Ambas secciones de protocormos fueron sembradas en medio MS modificado, en presencia de distintas concentraciones de ácido a-naftalenacético (ANA) (0, 0.1, 0.5, 1 mg/l) y bencila-denina (BA) (0, 1, 2 y 3 mg/l).

Con un microscopio estereoscópico se cuantificó el número de PLB´s formados por explante para cada tratamiento. Los datos obtenidos fueron sometidos a un análisis de varianza de una vía (ANOVA) y se aplicó un análisis de Tukey y Kramer (SAS 2000) para determinar las diferencias de medias estadísticamente significativas entre tratamientos. Después de 165 días de cultivo, los PLB's obtenidos fueron individualizados y subcultivados en el mismo medio MS modificado pero sin reguladores de crecimiento con el fin de incrementar su talla hasta formar plantas completas. Las plantas obtenidas fueron colocadas en troncos de encino y tepozán para su aclimatización. Sobre las raíces se agregó una pequeña cantidad de *Sphagnum* y agrolita (proporción 3:1) y a su vez sobre éste fue colocada una porción de gasa adhiriéndola a los troncos con hilo de cáñamo.

Resultados y Discusión

La germinación de las semillas se registró a los 15 días después de realizada su siembra en el medio MS modificado y fue mayor al 90%. El porcentaje de germinación obtenido para *Euchile mariae,* es similar al reportado para diferentes géneros de orquídeas utilizando frutos aún no dehiscentes (George & Sherrington 1984). Hernández *et al*. (2001), reportan la germinación de semillas de *Laelia anceps* y *Catasetum intergerrinum* cercana al 100% en medio Knudson C a partir de cápsulas indehiscentes. En nuestros cultivos, se observó una hidratación de las semillas y ruptura de la testa seminal dando lugar a la formación de protocormos, en los que después de 30 días de cultivo se formaron hacia el ápice los primeros primordios foliares. Los protocormos obtenidos en esta etapa de desarrollo al ser seccionados en mitades apicales y basales y cultivados en medio MS modificado adicionado con distintas concentraciones de ácido α-naftalenácetico (ANA) y benciladenina (BA), presentaron una apariencia nodular que se hizo evidente a partir de los 30 días de cultivo (Fig. 1A). A los 65 días de cultivo los nódulos formados mostraron un cambio en su tamaño y morfología, éstos comenzaron a adquirir una forma redondeada semejante a la de un protocormo, denominándose a las estructuras formadas cuerpos parecidos a protocormos o protocorm like bodies (PLB's) que surgieron de manera

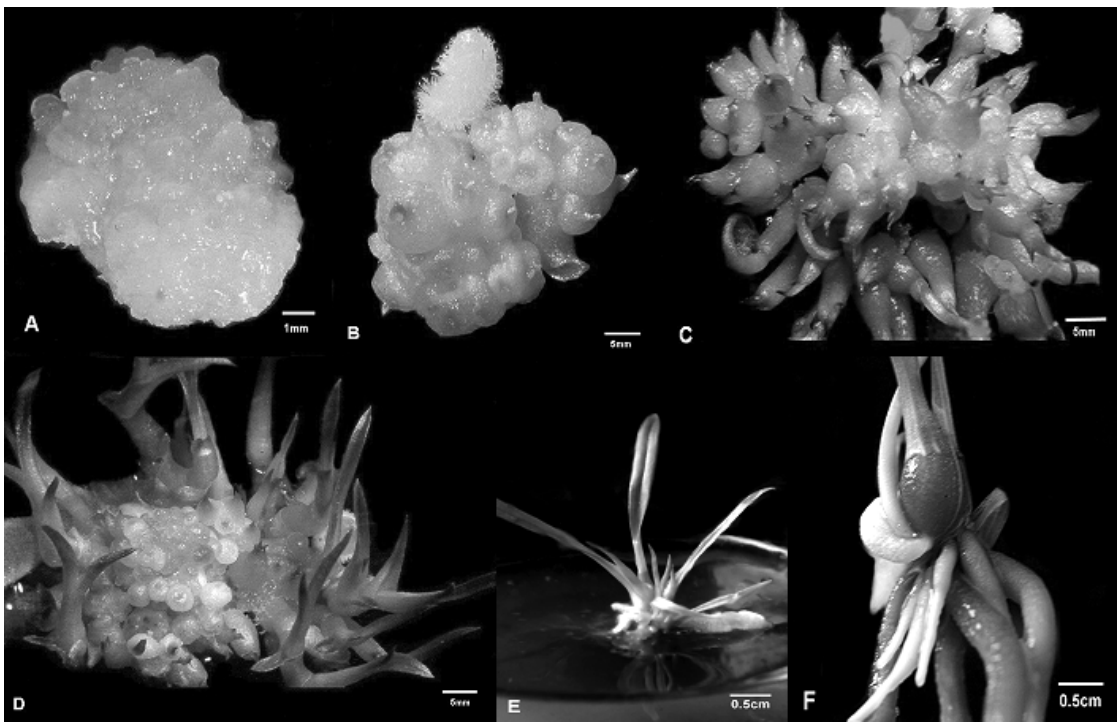

FIGURA 1. Formación de PLB´s y desarrollo de plántulas de *Euchile mariae* a partir del cultivo *in vitro* de secciones apicales y basales de protocormos en medio MS modificado. **A)** Desarrollo de nódulos en la superficie del explante a los 30 días. **B)** Formación directa de PLB's a partir del explante, a los 65 días. **C)** Elongación de los PLB's, a los 120 días. **D)** Desarrollo foliar y radicular, a los 165 días. Fotografías tomadas por Isabel Pineda Hernández.

directa del explante (Fig. 1B). A los 120 días de cultivo era notoria la elongación que estaba iniciando en los PLB's formados (Fig. 1C). A los los 165 días los PLB's presentaban hojas más desarrolladas y comenzó la aparición de la primer raíz (Fig. 1D).

La inducción de una respuesta morfogenética en *Euchile mariae*, a partir del cultivo de mitades de protocormos (apicales y basales) en medio MS modificado, fue diferencial respecto al número de PLB's formados. La mayor formación de PLB´s se registró a partir de las secciones basales de protocormos, en donde al termino del periodo de inducción (165 días de cultivo) se obtuvieron un promedio de 11.44 ± 9.64 PLB's por explante, mientras que para las secciones apicales se formaron en promedio 4.98 ± 5.33 PLB's por explante. Al cultivar las secciones apicales de protocormos, la mejor respuesta morfogenética se obtuvo en el medio MS modificado adicionado con 1 mg/l de ambos reguladores de crecimiento, donde se formó el mayor número de PLB's por explante (11.00 ± 9.55) (Tabla 1).

Resultados similares fueron observados en el cultivo *in vitro* de mitades de protocormos de *Laelia anceps* donde obtuvieron la mayor formación de brotes por explante en cultivos en medio Knudson C adicionado con 0.5 mg/l de BA y con 1 mg/l de ANA (Hernández *et al*. 2001). Así mismo, Mauro *et al.* (1994), sugieren utilizar altas concentraciones de BA y bajas concentraciones de ANA para lograr la formación de un gran número de brotes y PLB´s en *Cattleya aurantiaca* en medio MS, encontrando la máxima formación de PLB's utilizando 10 mg/l de BA en combinación con 0.1 mg/l de ANA.

En el caso de las secciones basales de protocormos, el mayor número de PLB's se formó en el medio MS modificado adicionado con 1 mg/l de BA, sin la adición de ANA, obteniéndose un promedio de 33.07 ± 20.75 PLB's por explante (Tabla 2). Nuestros resultados coinciden con los reportados por Baltazar (2004), quien al cultivar mitades de protocormos de *Oncidium tigrinum*, encontró que el mejor tratamiento para la formación de PLB's y su desarrollo a plán-

TABLA 1. Promedio de PLB's por explante obtenidos a los 165 días de cultivo, a partir de secciones apicales de protocormos de *Euchile mariae*, en medio MS modificado adicionado con distintas concentraciones de ANA y BA.

Tratamiento hormonal ANA/BA (mg/l)	N° total de PLB's	Promedio de PLB's por explante ± D.S
1/1	440	11.00 ± 9.55 a
0/3	388	9.70 ± 8.06 a
0.1/3	385	9.62 ± 11.79 a
1/3	348	8.70 ± 9.07 ab
0/1	199	4.97 ± 5.31 bc
0/0	183	4.57 ± 4.34 bc
0.5/3	176	4.40 ± 6.18 bc
0/2	149	3.72 ± 4.46 c
0.1/1	145	3.62 ± 3.29 c
0.5/1	127	3.17 ± 4.57 c
0.1/2	123	3.07 ± 2.67 c
1/2	100	2.85 ± 2.86 c
0.5/2	113	2.82 ± 3.67 c
0.1/0	102	2.55 ± 3.78 c
0.5/0	101	2.52 ± 2.91 c
1/0	98	2.45 ± 2.69 c

($p \leq 0.0001$). D. S.= Desviación estándar, PLB's = Cuerpos parecidos a protocormos.

TABLA 2. Promedio de PLB's por explante obtenidos a los 165 días, a partir de secciones basales de protocormos de *Euchile mariae*, en medio MS modificado adicionado con distintas concentraciones de ANA y BA.

Tratamiento hormonal ANA/BA (mg/l)	N° total de PLB's	Promedio de PLB's por explante ± D.S
0/1	1323	33.07 ± 20.75 a
0/0	954	23.85 ± 16.25 b
0.5/3	853	21.32 ± 15.61 b
0/3	823	20.57 ± 11.89 b
0.1/1	452	11.30 ± 9.75 c
0.1/3	435	10.87 ± 8.58 c
0/2	393	9.82 ± 11.08 c
0.1/0	317	7.92 ± 5.56 c
0.5/2	309	7.72 ± 8.24 c
1/0	296	7.40 ± 8.12 c
0.1/2	282	7.05 ± 6.79 c
1/1	232	5.80 ± 8.48 c
0.5/1	224	5.60 ± 8.92 c
0.5/0	155	3.87 ± 4.47 c
1/3	140	3.50 ± 5.20 c
1/2	122	3.48 ± 4.57 c

($p \leq 0.0001$). D. S.= Desviación estándar. PLB's = Cuerpos parecidos a protocormos.

tulas, fue el que presentaba solamente 1 mg/l de BA, obteniéndose a su vez en éste el porcentaje más elevado de sobrevivencia para los explantes.

La función del ANA al parecer no jugó un papel preponderante en la formación de PLB´s, mientras que la adición de BA al medio de cultivo favorece la formación de éstos tanto en las secciones apicales como basales de los protocormos. Taiz (1998), establece que la aplicación directa de citocininas al medio de cultivo estimula el crecimiento de yemas laterales en muchas especies, anulando el efecto inhibidor del meristemo apical de brote.

Una vez concluida la etapa de inducción, después de 165 días de cultivo, los PLB's obtenidos a partir de las secciones apicales y basales de protocormos de *Euchile mariae,* al ser individualizados y subcultivados en medio MS modificado sin reguladores de crecimiento, después de 60 días se desarrollaron hacia la formación de plántulas completas (Fig. 1E). A los 90 días, hacia la base del tallo se observó un ensanchamiento, que correspondió a la formación del pseudobulbo (Fig. 1F).

Las plantas obtenidas a partir del cultivo de ambas secciones de protocormos (Fig. 2A), fueron establecidas en condiciones *ex vitro* exitosamente en troncos de encino y tepozán (Fig. 2B), obteniéndose en ambos casos un porcentaje de sobrevivencia del 100% (Fig. 2C).

Los resultados obtenidos en este trabajo ofrecen una alternativa viable y de gran utilidad para la micropropagación de *Euchile mariae,* como una herramienta que permita reducir la presión que se

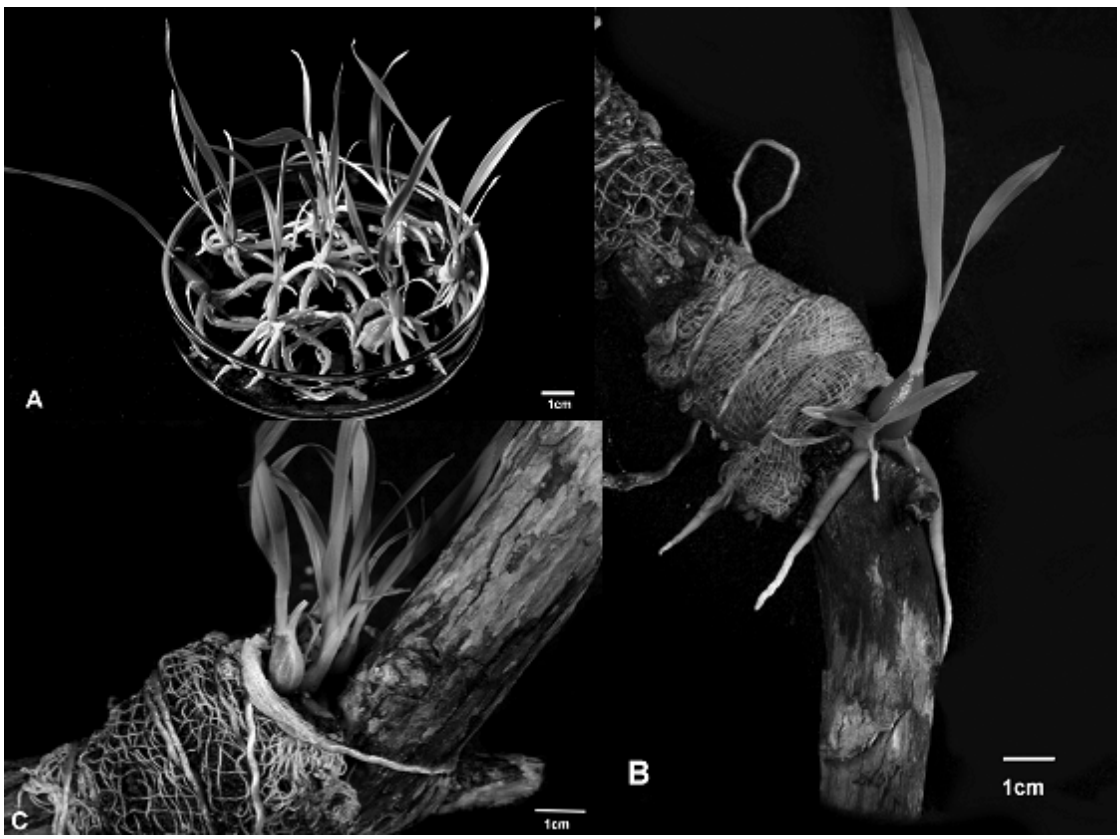

FIGURA 2. Establecimiento *ex vitro* de las plantas micropropagadas. A) plantas obtenidas a partir de mitades apicales y basales de protocormos de *Euchile mariae.* B) Montaje de las plantas micropropagadas sobre troncos de tepozán. C) Plantas de *E. mariae* después de 35 días en condiciones *ex vitro*. Fotografías tomadas por Mónica Vázquez Cortés.

ejerce en sus poblaciones silvestres y que a su vez, sirva como modelo para la propagación masiva de otras especies de orquídeas, que se encuentren amenazadas y/o en vías de extinción, contribuyendo a su conservación y aprovechamiento sustentable.

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LITERATURA CITADA

Arditti J. 1992. Fundamentals of orchid biology. John Wiley & Sons, Inc. USA. 691 p.

Ávila D.I. & K. Oyama. 2002.Manejo sustentable de *Laelia speciosa* (Orchidaceae). Biodiversitas 7 (43): 9-12.

Baltazar G.R. 2004. Micropropagación de *Oncidium tigrinum* Llave & Lex. (Orchidaceae) a partir de protocormos. Tesis de licenciatura. Facultad de Biología. Universidad Veracruzana. Xalapa, Veracruz. 124 p.

Dressler R.L. 1981. The orchids. Natural history and classification. Harvard University Press. London. 332 p.

Dressler R.L. 1993. Phylogeny and classification of the orchid family. Dioscorides Press. Portland, Oregon. 313 p.

Espejo S.A., C.J. García, F.A.R. López, M.R. Jiménez & S.L. Sánchez. 2002. Orquídeas del estado de Morelos. Orq. (Méx.) Vol. 16. Número único. 332 p.

George E.F. & P.D. Sherrington.1984. Plant propagation by tissue culture. Handbook and directory of commercial laboratories. Exegetics Ltd. Eversley, Basingstone, England. 709 p.

Hágsater E., M.A. Soto-Arenas, G.A. Salazar-Chávez, R. Jiménez-Machorro, M.A. López-Rosas & R.L. Dressler. 2005. Las orquídeas de México. Instituto Chinoín. México. 304 p.

Hernández H.J., S.O. Hernández & R.M. Mata. 2001. Regeneración de plántulas a partir del cultivo *in vitro* de mitades de protocormos de *Laelia anceps* Lindl. y *Catasetum intergerrimum* Hook. Amaranto 14 (1) : 3-12.

Knudson L. 1946. A new nutrient solution for the germination of orchid seeds. Am. Orchid Soc. Bull. 15 : 214-217.

Magaña P. & J.L. Villaseñor. 2002. La flora de México.

¿Se podrá conocer totalmente? Ciencias 66 : 24-26.

Mauro M., D. Sabapathi & R.A. Smith. 1994. Influence of benzylaminopurine and alpha naphthalenacetic acid on multiplication and biomass production of *Cattleya aurantiaca* shoot explants. Lindleyana 9 (3) : 169-173.

Mittermeier R.A. 1988. Primate diversity and the tropical forest. Case studies from Brazil and Madagascar and the importance of the megadiversity countries. Pp. 145-154 *in*: E.O. Wilson (ed.). Biodiversity. National Academy Press. Washington, D. C.

Murashige T. & F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 : 473-497.

Ramamoorthy T.P., R.Bye, A. Lot & J.Fa.1993. Biological diversity of Mexico: origins and distribution. Oxford University Press. New York. 812 p.

SEMARNAT. 2002. Norma Oficial Mexicana NOM-059-ECOL-2001. Protección ambiental-especies nativas de México de flora y fauna silvestres-categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-lista de especies en riesgo. Diario oficial (6 de marzo 2002). México, D.F.

Soto A., M.A. 1996. México. Pp. 53-58 *in*: IUCN/SSC Orchid specialist group. Orchids-status survey and conservation action plan. IUCN, Gland Switzerland and Cambridge, UK.

Soto A., M.A. 2002. *Euchile mariae* (Ames) Withner. *In*: E. Hágsater & M. Soto (ed.), Icones Orchidacearum. Orchids of Mexico Part 2,3. Asociación Mexicana de Orquideología. A.C. Mexico. Lámina 584.

Soto A., M.A. & G.A. Salazar. 2004. Orquídeas. Pp. 271-295 *in*: A.J. García-Mendoza, M.J. Ordóñez & M. Briones-Salas (eds.). Biodiversidad de Oaxaca. Instituto de Biología, UNAM-Fondo Oaxaqueño para la Conservación de la Naturaleza-World Wildlife Fund. México.

Taiz L. & E. Zeiger. 1998. Plant physiology. Second edition. Sinauer Associates, Inc., Publishers. Sunderland Massachusetts. 690 p.

Toledo V.M. 1994. La diversidad biológica de México. Nuevos retos para la investigación en los noventas. Ciencias. 34: 43-59.

UICN/SSC Orchid Specialist Group. 1996. Orchids-status survey and action plan. IUCN, Gland Switzerland and Cambridge, UK. 153 p.

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DETERMINACIÓN HISTOLÓGICA DE REGENERANTES DE *EUCHILE MARIAE* (AMES) WITHNER, (ORCHIDACEAE), OBTENIDOS A PARTIR DE PROTOCORMOS CULTIVADOS *IN VITRO*

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ABSTRACT**.** From the germination of seeds of *Euchile mariae* in modified MS medium, the formation of protocorms was achieved. Once these reached an average size from 2 to 5 mm long and the formation of his first leaf primordium, they were used like explants to induce a morphogenetic response. Through *in vitro* culture of top and bottom protocorms sections, were obtained differentiated structures from asexual origin. Their morphology was similar to protocorms obtained from the germination of seeds, in this way we call them protocorm like bodies (PLB's). Through of the histological analysis of these structures it was possible to reveal that these PLB's turned out to be somatic embryos. The histochemical tests demonstrate the presence of cellular contents like: proteins, lipids and starch; both in the cells of the embryos as well as in the cells of initial tissues.

PALABRAS CLAVE: orquídeas, cultivo de tejidos, protocormos, embriogénesis somática, histología, histoquímica

Introducción

Euchile mariae (Ames), Withner es una de las orquídeas endémicas de México que en la actualidad se encuentra catalogada en la Norma Oficial Mexicana (NOM-059-ECOL-2001) como una especie amenazada (SEMARNAT 2002). Se distribuye en los estados de Tamaulipas, San Luis Potosí, Hidalgo, Guanajuato, Querétaro, Puebla y Veracruz, en México, a una altitud de 800 a 1350 metros sobre el nivel del mar (Soto 2002). Es una especie epífita apreciada como ornamental, motivo por el cual ha sido colectada de manera excesiva e ilegal y aunado a esto ha habido una disminución de sus poblaciones silvestres por la destrucción constante de su hábitat.

Los patrones de desarrollo en las plantas han sido estudiados a través de la interpretación histológica de su estructura, topografía y arquitectura del desarrollo llegando a establecerse una caracterización anatómica de sus tejidos y estructuras que presentan las plantas desde su embriogénesis, organogénesis hasta su conformación en una planta reproductivamente madura caracterizando cada una de estas etapas y conociendo las modificaciones o cambios en los contenidos celulares durante estos procesos de desarrollo. Esto es posible a partir de la aplicación de diversas técnicas histológicas e histoquímicas. Las técnicas histológicas hoy en día son muy utilizadas en el cultivo *in vitro* para la determinación de la vía de desarrollo de los regenerantes, lo cual es de suma importancia para el entendimiento de los sistemas *in vitro* (Yeung 1999). Sin la aplicación de estas técnicas, resulta imposible determinar con certeza la vía de desarrollo obtenida. Por otro lado, resulta difícil especular acerca de la respuesta posible de un explante sin el conocimiento previo de las características de las células involucradas en el proceso regenerativo.

El propósito de este trabajo fue determinar la identidad de los regenerantes formados a partir de secciones apicales y basales de protocormos cultivadas *in vitro*, así como conocer los procesos de desarrollo que se llevaron a cabo.

Materiales y método

Se fijaron en Navashin muestras de los regenerantes formados de las secciones apicales y basales de protocormos a los 35, 65 y 120 días de su cultivo *in*

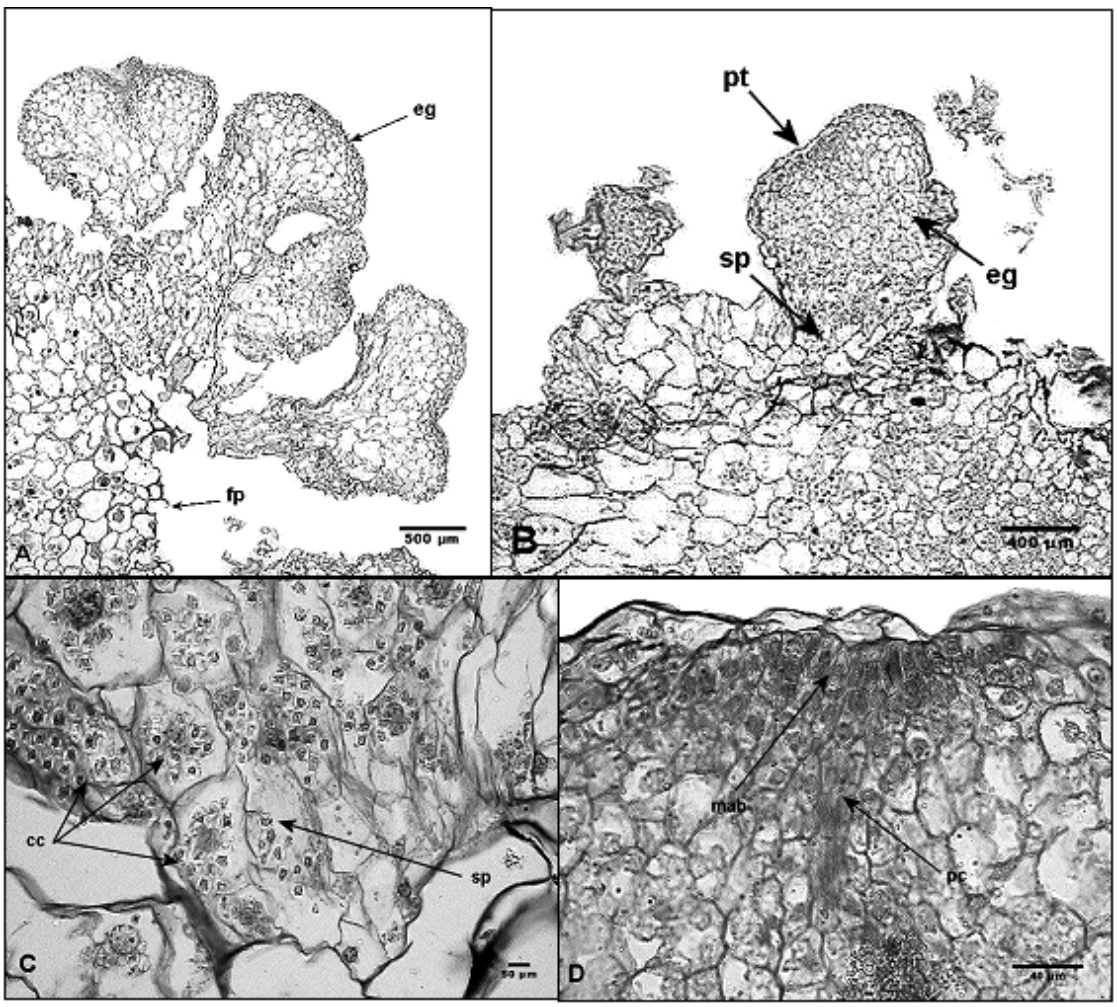

FIGURA 1. Secciones longitudinales de estructuras globulares, obtenidas a partir del cultivo *in vitro* de secciones de protocormos de *Euchile mariae* en medio MS modificado. **A)** Formación de estructuras globulares a partir de las células epidérmicas del tejido inicial. **B)** Formación de protodermis hacia la periferia y del suspensor hacia la zona basal. **C)**Suspensor y contenidos celulares. **D)** Formación del meristemo apical de brote y diferenciación del procambium. Fotomicrografías tomadas por la M. en C. Estela Sandoval Zapotitla. *Abreviaturas*: fp = fracción protocormo, eg = estructura globular, pt = protodermis, sp = suspensor, cc = contenidos celulares, mab = meristemo apical de brote, pc = procambium.

vitro en medio MS modificado (Murashige & Skoog 1962). A través de las técnicas histológicas convencionales, se procesaron estas muestras deshidratando en series graduales de agua-alcohol etílico-alcohol butílico terciario (ABT), en las siguientes concentraciones: 35%, 50%, 60%, 70%, 85%, 95% y ABT absoluto, infiltrando e incluyendo en parafina histológica (58 ºC). Las secciones histológicas se realizaron con un micrótomo de rotación American Optical 820, a 5 µm de grosor. Se realizó una doble tinción con safranina-verde rápido. La toma de fotomicrografías, así como la observación de las estructuras y tejidos se realizó con un fotomicroscopio Carl Zeiss-Axioskope. Se hicieron pruebas histoquímicas para la detección de contenidos celulares.

Resultados y discusión

La similitud morfológica de las primeras etapas del desarrollo, de las estructuras obtenidas con respecto a los protocormos de esta misma especie, nos permitió definirlos como PLB's. Mediante el análisis histológico de estas estructuras se pudo constatar que estos

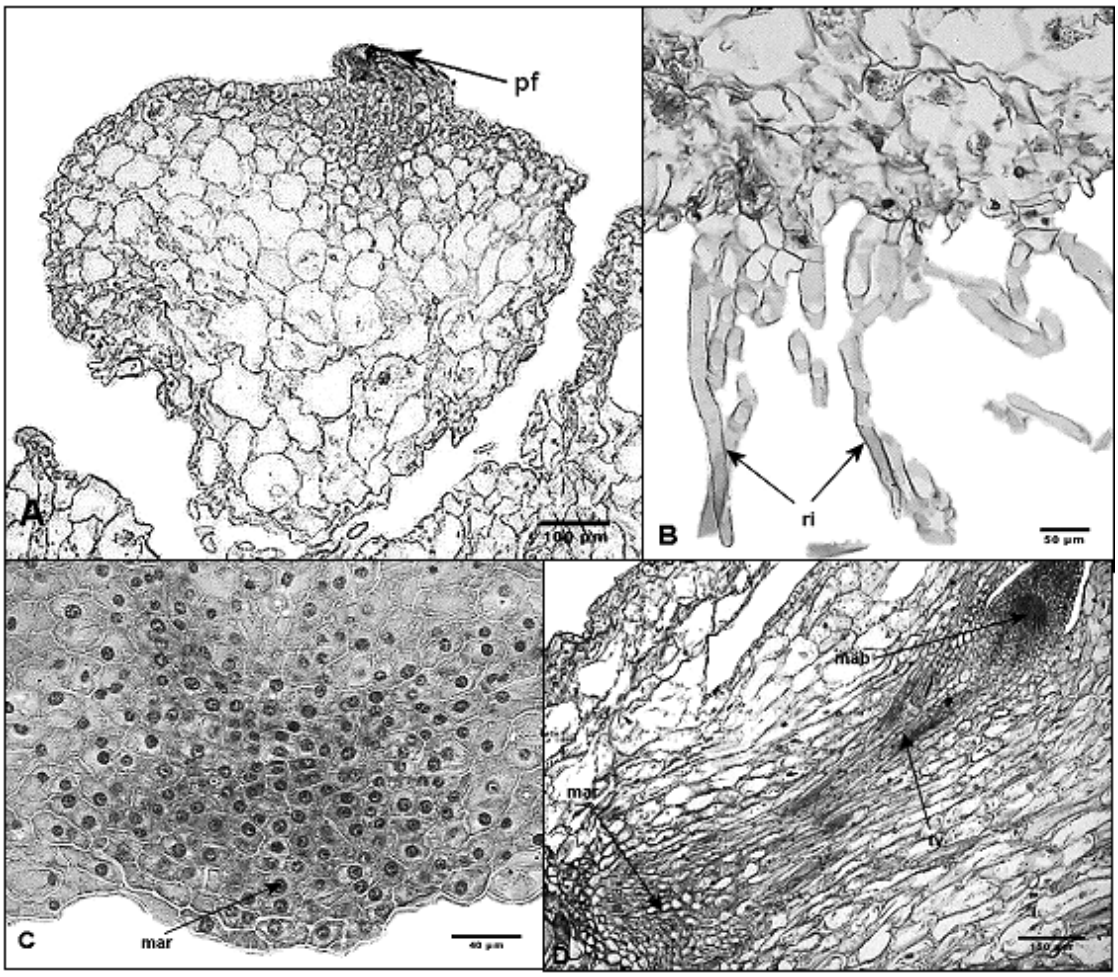

FIGURA 2. Desarrollo de plántula de *Euchile mariae,* a partir del cultivo *in vitro* de secciones de protocormos, en medio MS modificado. **A)** Formación del primer primordio foliar. **B)** Surgimiento de rizoides hacia la zona basal. **C)** Meristemo apical de raíz. **D)** Conexión del tejido vascular entre el meristemo apical de brote y el meristemo apical de raíz. Fotomicrografías tomadas por la M. en C. Estela Sandoval Zapotitla. *Abreviaturas*: pf = primordio foliar, ri = rizoides, mab = meristemo apical de brote, mar = meristemo apical de raíz, tv = tejido vascular.

PLB's resultaron ser embriones somáticos, por lo que la vía morfogenética seguida bajo las condiciones de este cultivo, fue embriogénesis somática directa.

A los 35 días de cultivo, las secciones histológicas obtenidas de los regenerantes mostraron la presencia de estructuras globulares en distintas etapas de desarrollo, que surgieron de manera directa a partir de las células epidérmicas del tejido inicial (secciones apicales y basales de protocormos) (Fig. 1A). Estos resultados concuerdan con los reportados por Morel (1974), quien al cultivar *in vitro* secciones de protocormos de *Cymbidium* en un medio nutritivo, observó la formación de nuevos protocormos a partir de la región epidérmica. Hacia la periferia de las estructuras globulares identificadas, se observaron células en un estrato definido, evidenciando la formación de la protodermis y hacia la zona basal de éstas se observó un adelgazamiento y reducción de tamaño que muestra la formación del suspensor (Fig. 1B). Se encontró una polaridad de contenidos celulares, siendo éstos menos abundantes hacia la región apical y más abundantes hacia la región basal de los embriones somáticos y hacia la zona del suspensor (Fig. 1C). Hacia la región apical, se hizo evidente una zona meristemática, que corresponde al meristemo apical de brote, constituido por células pequeñas, densamente teñidas, en activa división celular y con una alta relación núcleo-

citoplasma (Fig. 1D). Posteriormente, comenzó a hacerse evidente una diferenciación celular interna como una columna central constituida por células densamente teñidas, de forma alargada lo que se define como el procámbium (Fig. 1D), el cual dará lugar a la formación del tejido vascular.

A los 65 días de cultivo, en la parte dorsal de las estructuras globulares formadas, se observó la formación de el primer primordio foliar (Fig. 2A), y hacia la región basal se observó la formación de rizoides (Fig. 2B). A los 120 días de cultivo en posición opuesta al meristemo apical de brote comenzó a evidenciarse otra zona meristemática que correspondió al meristemo apical de raíz (Fig. 2C), formado por células pequeñas, esféricas, con citoplasma denso y núcleos prominentes. Este meristemo continúo su desarrollo y dio lugar a la formación de la primera raíz. Fue posible observar la conexión del tejido vascular entre ambos meristemos formados (Fig. 2D). Muchas especies de orquídeas presentan un patrón de desarrollo similar al encontrado para *Euchile mariae,* el cual también es semejante al documentado por Nishimura (1981), para *Cattleya aurantiaca,* y por Leroux *et al.* (1995) para *Cypripedium acaule,* durante el desarrollo del embrión hasta la formación de una planta completa.

Mediante la aplicación de colorantes específicos que reaccionan y producen coloraciones determinadas, es posible reconocer microscópicamente la presencia de distintas sustancias presentes en los tejidos vegetales ya sean como metabolitos primarios, secundarios, formando parte estructural de la célula o como sustancias de reserva (Sandoval 2005). Con las pruebas histoquímicas realizadas, a los 35 días de cultivo se detectó la presencia de contenidos celulares, tales como: proteínas (azul negro de naftol), lípidos (sudán IV) y almidón (lugol); estos contenidos se encontraron al interior de las células del tejido inicial (secciones de protocormos), así como en las células de los embriones. De los tres contenidos celulares identificados en los embriones somáticos de *Euchile mariae*, el más abundante fue el almidón, mientras que, las proteínas y lípidos se encontraron en menor cantidad.

En la presente investigación se reportan las diferentes etapas del desarrollo de los embriones somáticos obtenidos a partir de secciones apicales y basales de protocormos hasta su formación en una planta completa. Este es uno de los pocos estudios a nivel global, que determina fehacientemente la identidad de los regenerantes.

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LITERATURA CITADA

Leroux G., D. Barabé & J.Vieth. 1995. Morphogenese comparee de protocormes du *Cypripedium acaule* (Orchidaceae) cultivés *in vitro* avec ou sans sucre. Can. J. Bot. 73 : 1391-1406.

Morel, G.M. 1974. Clonal multiplication of orchids. In: Withner, C. L. (ed.). The orchids. Scientific studies. Wiley-Interscience. USA. 604 p.

Murashige T. & F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 : 473-497.

Nishimura G. 1981. Comparative morphology of *Cattleya* and *Phalaenopsis* (ORCHIDACEAE) seedlings. Bot. Gaz. 142 (3) : 360-365.

Sandoval E.Z. 2005. Técnicas aplicadas al estudio de la anatomía vegetal. Cuadernos 38. Instituto de Biología, UNAM. 278 p.

SEMARNAT. 2002. Norma Oficial Mexicana NOM-059-ECOL-2001. Protección ambiental-especies nativas de México de flora y fauna silvestres-categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-lista de especies en riesgo. Diario Oficial (6 de marzo 2002). México, D.F.

Soto A., M.A. 2002. *Euchile mariae* (Ames) Withner. *In*: E. Hágsater & M.A. Soto A. (eds.), Icones Orchidacearum. Orchids of Mexico Part 2,3. Asociación Mexicana de Orquideología. A.C. México. Lámina 584.

Yeung, E.C. 1999. The use of histology in the study of plant tissue culture systems- some practical comments. In Vitro Cell Dev. Biol. Plant 35 : 137 – 143.

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YOUNG ADVENTURES IN ORCHID CONSERVATION

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RESUMEN. Esta conferencia decribe el trabajo empezado por un Colegio Británico único, que se ha concentrado intensamente en el campo de la conservación de las orquídeas para crear el Proyecto de Orquídeas del Colegio Writhlington. A través de la perspectiva de uno de los estudiantes con más experiencia en el proyecto, se analizan los resultados de los últimos siete años del proyecto, para ver como y porqué el proyecto ha alcanzado su situación actual. Se indican tambien las finalidades y objetivos futuros del proyecto. La conferencia subraya el enfoque del proyecto hacia una conservación efectiva en el Reino Unido y afuera, así como el envolucramiento de una comunidad tanto a nivel local como en una escala global. Se exploran también la experiencia educativa y el contexto curricular del proyecto.

KEY WORDS. Orchidaceae, conservation, threats, tropical research, micro-propagation, *ex-situ* conservation, science, enterprise.

Introduction

The Writhlington School Orchid Project has been described as a world leader in Orchid Conservation. Where Science, Enterprise and Conservation has been combined to a point where they are the future forerunners in conservation. Writhlington School is a state secondary school with 1200 pupils aged between 11 and 18. The Orchid project was project was started when an orchid grower donated a collection of hybrid *Cymbidiums* for the school greenhouses. These greenhouses were little used and dilapidated until they came into the management of Simon Pugh-Jones; the schools head of Physics. Simons Passion for orchids came from childhood inspiration from working at Keith Andrew Orchids, an orchid nursery in Dorset.

It has been in the last six years that 'The Project', as it is more commonly known, has established it self nationally and internationally. Presently we operate with a productive Orchid propagation laboratory. This has been constructed within one of the schools unused female toilets, which is highly practical in terms of the existing fixtures, and is now transformed into the hub of our conservation work. Internationally we work with groups in Guatemala, Sikkim (India), Laos and Costa Rica. And we now have developing partnerships in Gabon (Africa), Belize and Turkey. Nationally we work with a range of institutions and commercial organisations. Scientifically we work with RBG Kew, both with Orchid Taxonomist, Dr. David Roberts, and extensively with Kew's micro propagation department with the assistance of Grace Prendergast (fig. 1). Our entrepreneurial partners are The Eden Project, @t Bristol, and Bicton Park Botanical Gardens. On the horticultural side we have numerous links with the Royal Horticultural Society (RHS).

WHEN I ARRIVED AT THE SCHOOL. I arrived at Writhlington School in September 2000, and instantly took to the extra curricular club known as 'the Greenhouse Club'. I have always had an active interest in Horticulture. Some of my earliest memories are

FIGURE 1. Callum, 13, working with at Bristol plant experts.

of me in my Grandfather's greenhouse tending to the tomatoes that he grew. Generally this meant me eating them. After entering the school Greenhouse, I was bemused by these 'Orchids', with their strange looking flowers and even weirder names. The parts that particularly inspired and fascinated me about these unique plants were all extraordinary places they grew. I was immediately filled with visions of tropical rainforest, stretching from Brazil to Burma and Venezuela to Vietnam. I was instantly hooked, and pretty soon decided to make a career out of my favourite hobby. In the six years since arriving at the school I have been involved with almost all the branches of the project. I have constructed numerous prize winning displays at both local and national Orchid shows, spending many hours in the lab and I have produced research working with both botanical and scientific institutions.

Once I had joined the club I was immediately put in charge of the genera within the Oncidiinae family. After a few weeks I had learnt the basics of the family and how to grow these Oncidiums and Odontoglossums, I was able to tell visitors where they grew, what temperatures they prefer. And I was starting to get my tongue around pronouncing these ridiculous names, like *Oncidium ornithorynchum* and *Osmoglossum pulchellum.* After a few months I was allowed to make my first hybrid orchid, I selected two of my plants, and with the assistance of a match stick made the hybrid *Colmanara* Hawaii x *Miltonia cuneata*. I remained in charge of my section within the greenhouse for a total of five years, till they were eventually passed on to one of the younger members of the greenhouse club. Soon I relished that there was more to the orchids than just Miltonias, and eventually started to learn more and more about the other genera and tribes. I became particularly interested in Cymbidums, Dendrobiums, and Phragmipediums. Soon my obsession for orchid made its way into my home. I started growing orchids at home with just one hybrid *Oncidium*, but soon they began to dominate my bedroom. Currently I grow over 300 orchids within my house, ranging from small seedling species growing in my bedroom window sill, to the larger specimen plants which have made there habitat in my living room. I currently specialise in growing *Phragmipedium*.

FIGURE 2. Working in a laminar air flow cabinet.

FIGURE 3. Writhlington school propagation lab.

It was after the construction of the orchid lab at the school, that the project really begin to take off. We received our first laminar air flow cabinet (fig. 2, 3) from the Bristol eye hospital and seed sowing began. Soon we were donated a further three airflows from the RBG Kew micro propagation department, and we were able to convert the schools disused female toilets into a

fully functioning propagation lab. It was in the year of 2004 that I took over the day to day running of the propagation lab, and I awarded myself the grand title of Lab manager. After three years of trial and error, the project had one of Britain's largest Orchid specialist propagation labs in terms of number of orchids sold annually.

In the year of 2005 I was lucky enough to be including on two tropical rainforest research trips. In August I travelled as part of a group of four to the Peten region of Guatemala (fig. 4). We were working with a group of Orchid Conservationists known as Yaxhà Orchids. On this expedition we were faced with Crocodiles, Mayan Architecture, and also the painful end of a swarm of tropical hornets. With the assistance of Federico Fahsen we spend two weeks in primary seasonally dry rainforest, conducting vital research on orchid diversity over the reserve. This work was eventually published, and display at the RHS London Orchid Show where it receive a RHS Gold medal (fig. 5). And it was also presented at the 'Young Scientist of the Year' competition, hosted by the Royal Society. I have also spent two weeks working in the Mata Atlantic rainforest, working with the Rio Atlantic trust (fig. 6). I used this expedition as a chance to test my theory on orchid diversity, developed while in Guatemala.

After returning from my first expedition I was approached to give a lecture to a local gardening society. Since then I travelled across the country lecturing to enthusiast, gardeners, and orchid fanciers. This has given me a chance to both improved my self confidence and allowed me a chance to deliver the message of the Writhlington School Orchid Project.

THE DEVELOPMENT OF THE ORCHID STARTER KIT. One of the key turning points in the success of the Writhlington School orchid project has been the development of our unique product, known as the Orchid Seedling conservation kit(fig. 7). Initially invented by the 2003 Young Enterprise Company, Stem Labs, the kits has gone through serious changes to become the projects largest selling product; with sale reaching over £8,000 a year. The Kit consists of a seedling orchid, growing in-vitro on a nutrient agar jelly, a pot and potting media, for growing the orchid. We have also developed instructions for effective growth of the seedling orchid, and offer free advice through our website.

FIGURE 4. August 2005 expedition team to Guatemala, shores of Laguna Yaxhà.

FIGURE 5. 2006 London Orchid show, RHS Gold medal.

FIGURE 6. October 2005 expedition team to Brazil.

Currently we make the majority of our sales through the whole sale market (fig. 8). We sell through The Eden Project, Bicton Park Botanical Gardens @t Bristol, Hillier's Garden Centres, and we have a provisional agreement to sell our kits through

FIGURE 7. Orchid seedling conservation kit.

FIGURE 8. Sales area of orchid seedlings in Bicton Park Botanical gardens.

the Kew shop. The kits are individually branded to the organisation that they are distributed to, and £1 from every kit sold is sent back to the community that we work with.

Our Methods of Conservation

After seven years we can identify some key ways in which effective conservation can be introduced and maintained. These point have been identified as,

- Working with the local community
- Identifying flagship species
- Our media appeal
- Our links to specialists
- Our unique position
- Ability to be self sustaining
- Counterpart organisations
- Provide real rewards

I will consider each of these in turn.

WORKING WITH A LOCAL COMMUNITY. In each of the countries that we work, we try to link our scientific expedition with a local school. By doing this we can help in educate the community, raising the issues of conservation, and demanding its significance. We aim to link each school to a counterpart organisation in the U.K. setting a positive agenda for the future

IDENTIFYING FLAGSHIP SPECIES. Through identifying a flagship species, which can either be a well known cultivated species, such as *Prosthechea cochleata*, or a completely new species, we can centre each project on the sale and conservation of these flagships. We try to identify orchids which are both visually attractive to a buying customer, but must also have an interest factor about it; such as unique characteristics. Examples include our flagship in Sikkim, *Cymbidium whiteae,* which is endemic to the mountains surrounding Gantok. Some people estimate that there are less than fifty of these plants left growing in the wild. We have raised many thousands of these seedlings in our lab and will take them back to Gantok for distribution to locals, relieving pressure of the illegally collected plants and involving local gardeners in the conservational loop. We found seed of this orchid from a cultivated plant in the U.K.

OUR MEDIA APPEAL. Because we are a school, the media has taken to the project extremely well. This has enabled us to advertise what we do for free. This means that nationally we are well known in both the Orchid community, as well as with the non enthusiasts. We have appeared in on both television, and in the newspapers. By getting the nation behind us, from coverage such as the RHS Chelsea flower show, we can increase sales and support for orchid conservation.

SPECIALIST LINKS. Over the last six years we have made links with countries and organisation that no other school project has been able to do before. Our students communicate with world experts in other countries about there individual research. We have links with botanical gardens, universities, and scientists globally.

UNIQUE POSITION. We are in a unique position; because we are a school. With our position at the heart of the local community and our army of young volunteers we have the opportunity to do things that are not possible for many conservation groups. Each of the students

involved are able to complete up to 50% of there science coursework based around the orchid project and can enter into careers in Horticulture, Botany and Ecology. Students take part in expeditions to tropical forest gaining personal skills and broadening the knowledge base of the project.

ABILITY TO BECOME SELF SUSTAINING. Because we are a Business and Enterprise specialist school we are able to fund all our work in tropical countries through the sale of the products from companies within the school, run by the students. Through programs such as Young Enterprise we have been placed in a position where we are almost completely self funding. It also gives our students a real chance to see how business works, whilst gaining real rewards such as places on our expeditions. In 2006 Writhlington was awarded 'Enterprise school of the year'

COUNTERPART ORGANISATIONS AND PROVIDING REAL REWARDS. With each tropical country and community we work with, we try to link it with a counterpart organisation in Britain. For we have example linked Yaxhà Orchids in Guatemala to the Bicton Park Botanical gardens in Devon. The idea being that funds raised from U.K. sale of our orchid starter kits can be sent out to support work in Guatemala. Bicton Parks gains through information and links with Yaxhà.

What is the future of the Writhlington School Orchid Project?

Currently we see a number of routes for the future of the project. Ideally we hope to continue our current trend with work in tropical countries. We hope that our orchid conservation kits will continue to sell with the popularity that they have today, and will continue to fund all our work with orchid research. It is also hoped that with the development of a new school at Writhlington, planned for September 2010, our decrepit greenhouse can be replaced with a state of the art visitor centre and educational growing area. And we also hope to go on with developing new links, as well as maintaining existing ones. At the moment we have a number of both established and developing links with partners in tropical regions.

PAKSONG ORCHID PROJECT. We are currently working in Laos to find a way to protect the native orchids from the Chinese medicine trade, and also the Thai cut flower market. We are working with the community in Paksong to identify potential objectives for effective conservation and rural development. Our plan is to relieve pressure on threatened wild populations by encouraging coffee farmers to grow orchids on and under their coffee trees. These orchids will be raised from seed in a new Lab in Paksong. As well as supplying the existing markets with sustainable crops, it will provide the low income coffee farmers with a second crop. Local orchid species under threat include *Dendrobium compactum,* which is illegally harvested by the tonne, and is exported to China.

GABON ORCHID PROJECT. This project was launched earlier this year, and aims to fund the employment of staff for the Monte Crystal National park. We will be selling orchids such *Ansellia africana* through the Hiller chain of garden centres. We are also working with school iniatives in South Africa.

THE ORCHID ICE CREAM PROJECT. Working as part of a committee, with the RHS, the Eden Project and Turkish partners, we are operating to find horticultural solutions to the unsustainable collection of terrestrial orchid tubers in Turkey (fig. 9). These tubers are boiled, dried, and then ground up to make a powder known as salep; which is used to flavour ice cream, also known as Dondurma.

What are the lessons that can others learn from the

FIGURE 9. Committee of the Turkish orchid ice cream project.

Writhlington Experience? We have identified the key points for success to be:

- Aiming high
- Making links

We have demonstrated that an ordinary state school can have a major impact in conservation while delivering quality educational experiences, but for this to happen the support of our partner organisation has been essential.

Oh, and by the way all this has been great fun for me and at 17 years old I feel ready for an exiting career in plants and conservation.

ACKNOWLEDGMENTS. I would like to thank Simon for giving me this chance to do something worth while at school. With out all the opportunities the project given me, I wouldn't be in the position that I am in today. I would also like to thank him for putting up with me on all our expeditions. I would also like to thank the Stanley Smith horticulture trust for sponsoring me to attend this conference. Without there generous support I would never have been able to make this trip. I would like to show appreciation to the staff at Writhlington School for giving me the time off school, so that I could attend this conference. And for allowing me so many days of school over the last six years to allow me to work with the orchids.

Callum Swift is a sixth form student currently studying his A-levels at Writhlington Business & Enterprise specialist school, England. He has been involved with the Writhlington School Orchid Project for over six Years, and has plans for further work in orchid conservation after studying a Botany degree at University. He has extensive experience in the field of orchid conservation, and has work with experts from RBG Kew, and the Eden project; and has played a key role in the development of the micro propagation department within the Writhlington School Orchid project.

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ADQUISICIÓN DE COMPETENCIA PARA LA MICROPROPAGACIÓN DE *STANHOPEA TIGRINA, LAELIA ANCEPS, EPIDENDRUM VEROSCRIPTUM* Y *CATTLEYA* x ESBETTS (ORCHIDACEAE)

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ABSTRACT. Regeneration protocols were established from the in vitro culture of protocormos of *Stanhopea tigrina, Epidendrum veroscriptum, Laelia anceps* and *Cattleya* x esbetts using the Murashige and Skoog culture medium added with different concentrations of N^6-benzyladenine (BA) (0, 1, 3, y 5 mg/l) y 2,4 dichlorofenoxiacetic acid (2,4-D). The regeneration and formation of new plantlets was achieved by multiple shoot and also through protocorm like bodies (PLBs). For each species the best concentration of growth regulator, as well as the time of acquisition of competence for the highest shoot formation and/or PLBs were determined. As far as we know there are no reports with reference to the acquisition of competence within the Orchidaceae family that allow the production of many individuals within the shortest time and reducing the financial cost. This could form the base that covers their commercial demand and also contribute to the conservation and sustainable use of mainly wild species.

PALABRAS CLAVE: orquídeas, protocormo, adquisición de competencia, cultivo de tejidos, micropropagación

Introducción

La familia Orchidaceae es una de las más grandes y más diversas familias de plantas, se estima que existen aproximadamente 800 géneros y alrededor de 25,000 especies (Arditti, 1992; Dreesler, 1993; Espejo *et al*., 2002; Dixon *et al.,* 2003). Es una familia cosmopolita y prácticamente se distribuye en todo el mundo con excepción de los hielos permanentes y los desiertos (Quintanar, 1961). En México se distribuyen alrededor de 1,200 especies de orquídeas (Espejo *et al*., 2002), con un endemismo de 40%; y los estados con mayor diversidad de orquídeas son: Chiapas, Oaxaca, Veracruz, Puebla, Hidalgo, San Luis Potosí, Guerrero, Michoacán, Jalisco, Nayarit y Sinaloa (Soto, 1996). A pesar de ser una de las familias más grandes de plantas con flores, la distribución de las orquídeas ha disminuido notablemente en nuestros días, estos cambios se deben principalmente a la alteración y destrucción del hábitat, además muchas de las poblaciones silvestres están siendo seriamente afectadas por el volumen de individuos reproductores que se extraen para satisfacer la demanda comercial de que son objeto debido a que son altamente apreciadas como especies ornamentales. Lo anterior trae como consecuencia el lento o nulo restablecimiento de las poblaciones, debido a que presentan una baja tasa de crecimiento, ciclos de vida relativamente largos y escaso reclutamiento de nuevos individuos bajo condiciones naturales, llegando a ocasionar que un gran número de ellas se encuentren en peligro de extinción (IUCN /SSC Orchid Specialist Group 1996; Sosa y Platas, 1998; Ávila y Oyama, 2002).

La rareza y belleza de las orquídeas han atraído la atención muchos horticultores y/o coleccionistas, en muchos casos las poblaciones de diversas especies han sufrido sobrecolectas exhaustivas para satisfacer el comercio, éste tráfico ilegal de ejemplares silvestres ha aumentado principalmente desde la segunda mitad del siglo XX, teniendo como consecuencia que muchas poblaciones naturales hayan disminuido considerablemente, de hecho en algunos casos, poblaciones enteras han desaparecido (Betchtel *et al.*, 1981; IUCN/ SSC Orchid Specialist Group, 1996; Ospina, 1996; Hágsater y Soto, 2001). Los más afectados han sido las especies con alto potencial ornamental, entre alguno de los géneros que incluyen especies de alta importancia hortícola se encuentran: *Laelia* (por sus flores grandes y espectaculares), *Oncidium* (por la belleza de sus inflorescencias y la potencialidad de crear híbridos), *Rhynchostele* (por la belleza de sus

flores) *Encyclia* (por la sencillez de las mismas) y *Stanhopea* (por la rareza y extravagancia de sus flores) (Jiménez *et al.*, 1998).

La conservación de orquídeas no es una tarea fácil y principalmente en países tropicales como México, donde se encuentran la mayor diversidad de especies y es también donde la problemática es más grave. En México la alteración del hábitat y la sobrecolecta de orquídeas ha conducido a que 182 especies de orquídeas se encuentran dentro de algún estatus de conservación; se considera que una especie está extinta en el medio silvestre, 16 en peligro de extinción, 58 amenazadas y 107 sujetas a protección especial (SEMARNAT, 2002). En un gran número de mercados públicos en todo México existe una venta masiva de individuos colectados ilegalmente, en donde es posible encontrar especies en peligro de extinción, por lo que esta actividad se ha convertido en una de las principales causas de la disminución del número de individuos y poblaciones de las especies de orquídeas mexicanas (Hágsater & Soto 2001, Salazar 2003). En muchas ocasiones el uso que se le da a estas especies es únicamente durante el periodo de floración, una vez que la flor se marchita, es muy frecuente que la planta sea desechada. En otras ocasiones el poco conocimiento que la gente tiene sobre el cultivo de las plantas, hace casi imposible la supervivencia de la misma. Claros ejemplo de esta situación son las siguientes especies:

Stanhopea tigrina Bateman es una especie endémica de nuestro país, muy apreciada por su valor ornamental, lo que ha provocado una sobrecolecta de individuos silvestres y una comercialización ilegal (Soto 2002); actualmente se encuentra bajo protección especial por parte del gobierno federal Mexicano (SEMARNAT, 2002), por lo cual, es fundamental buscar alternativas para su conservación y preservación.

Laelia anceps Lindley es una especie endémica mexicana y una de las más utilizadas en hibridización (Halbinger y Soto, 1997), con un alto potencial ornamental y de gran interés económico; aunque no se encuentra catalogada dentro de la NOM-059-ECOL-2001, las presiones de colecta y comercio ilegal han disminuido considerablemente sus poblaciones, por lo que es preciso estudiar esta especie y ofrecer alternativas para su cultivo y comercialización.

El género *Epidendrum* está muy extendido en nuestro país, dentro de las especies que se distribuyen en México se encuentra *E. veroscriptum* Hágsater, a pesar que no se encuentra bajo alguna categoría de protección por parte del gobierno Mexicano, tiene un alto potencial ornamental y económico (García-Cruz y Sánchez, 1999), además que el hábitat donde se distribuye, el bosque mesófilo de montaña, es uno de los ecosistemas que están en riesgo de desaparecer y por consecuencia, las especies que contiene.

Finalmente, *Cattleya* x Esbetts es un híbrido muy apreciado como planta de ornato, de gran importancia comercial debido al tamaño, color y belleza de sus flores, popular por su fácil cultivo (Hágsater, 1971), el cual se puede comercializar por productores nacionales y generar recursos económicos importantes.

En este contexto, la conservación y uso sustentable de germoplasma valioso se ha planteado como una medida inaplazable y prioritaria; la conservación se puede englobar en dos grandes puntos: Se ha intentado realizar una efectiva conservación *in situ* a través del mantenimiento de áreas naturales protegidas, realizando programas de recuperación de especies, restauración de hábitats, control de especies invasoras y manejo de poblaciones de plantas y ecosistemas; sin embargo, cuando lo anterior no es posible llevar a cabo con éxito, o se pretende reforzar los esfuerzos realizados, se puede recurrir a la conservación *ex situ;* en este sentido los jardines botánicos y otras instituciones de investigación, tienen una importante función, buscando rescatar el germoplasma amenazado mediante el mantenimiento de colecciones vivas, estableciendo bancos de semillas, suministrando material para diferentes propósitos con el fin de eliminar o reducir la presión de colecta de que son objeto, cultivando aquellas especies con semillas recalcitrantes que no pueden ser mantenidas en bancos de semillas y a través de la propagación y multiplicación a través de las técnicas de cultivo de tejidos (Wyse, 2001).

El Cultivo de Tejidos Vegetales (CTV) es una herramienta biotecnológica cuyo fundamento se basa en la totipotencialidad celular, lo que permite mantener, en un medio de cultivo químicamente definido, cualquier estructura vegetal (embriones, semillas, inflorescencias, tallos, raíces, meristemos, células individuales, granos de polen, etc.) bajo condiciones asépticas controladas (George y Sherrington, 1984). Las técnicas de CTV han mostrado ser un importante procedimiento en la multiplicación, el mejoramiento y la conservación de las plantas útiles al hombre (Villalobos, 1985). En adición, tales técnicas han sido adaptadas para utilizarse con un amplio rango de

especies silvestres y han sido aplicadas exitosamente para la germinación en semillas y esporas, rescate de embriones, cultivo de meristemos y de callo (Fay *et al.* 1999), y han sido empleadas exitosamente en la propagación y conservación de especies en peligro de extinción (Rublúo 1985, Mata *et al*. 2001 a, b).

El principal uso de la micropropagación ha sido la multiplicación masiva de especies útiles para el hombre (Evans, 1990) y ha demostrado su utilidad práctica en especies de multiplicación deficiente o relativamente lenta como las orquídeas y en plantas que, aunque se pueden reproducir asexualmente mediante esqueje, estolón, bulbo, etc., su número se puede incrementar aún más cuando se propagan *in vitro,* como es el caso de las plantas de ornato, incluyendo a las orquídeas (Lozoya, 1985). Además de la propagación, con el CTV se pueden realizar estudios básicos de fisiología, genética, bioquímica, bioconversión y producción de compuestos útiles, asimismo se puede incrementar la variabilidad genética, además de que es posible realizar una conservación e intercambio de germoplasma más eficientes (Mroginski y Roca, 1993).

Para poder realizar una efectiva conservación *ex situ* de las orquídeas y desarrollar programas de uso sustentable adecuados, es factible recurrir a las técnicas del Cultivo de Tejidos Vegetales, que pueden ser utilizadas ampliamente para la propagación y conservación de germoplasma en riesgo o de alto valor ornamental y que han mostrado su efectividad con diversas especies en peligro de extinción (Fay, 1994, Fay *et al.* 1999, Serna, 1999, Mata *et al.* 2001b). Así se tiene que muchas orquídeas epífitas y algunas terrestres son cultivadas en ambientes artificiales, y no es inusual que se tengan cientos o miles de individuos creciendo en espacios limitados (Soto 1996). Actualmente hay una cantidad considerable de información acerca del mantenimiento *ex situ* de orquídeas vía cultivo de tejidos (Soto 1996). A nivel género se tienen reportados para *Laelia* trabajos sobre micropropagación (Mata y Salazar 2003); en el caso de *Epidendrum* se han utilizado secciones nodulares (Stewart & Button 1976), raíces (Churchill *et al.*, 1972), hojas (Churchill *et al.* 1973), y protoplastos (Yasugi *et a*l. 1986, Oshiro & Steinhart 1991); para *Cattleya* la literatura es muy amplia, con reportes sobre el uso de meristemos apicales (Morel, 1964; 1970), brotes laterales (Reinert y Mohr, 1967), secciones de hoja (Champagnat *et al.*, 1970; Arditti, *et al.*, 1971; Fu, 1978; Torres & Mogollón 2002), protoplastos (Capesius & Meyer 1977; Oshiro & Steinhart 1991) plántulas (Adelberg *et al*., 1998, Krapiek *et al*. 2003) y ápices caulinares (Torres & Mogollón 2000).

Por otra parte, en diversos estudios sobre regeneración *in vitro* de tejidos de plantas, principalmente leñosas, se ha descrito un evento de desarrollo denominado adquisición de competencia (Ellis & Bilderback 1989, Lo 1997, Sánchez-Espinosa *et al*. 2000, Azcón-Bieto & Talón 2001, Dhaliwal *et al*. 2003), el cual se define como el potencial endógeno que tiene una célula o tejido para responder a una señal organogénica, la cual activa una ruta particular de diferenciación para desarrollarse de una manera específica a través de un tipo de programación interior o "memoria" (Christianson & Warnick, citado por Ellis 1989, Hartmann *et al.* 1990; Lackie 1999, Segura, 2001, Taiz & Zeiger 2002). Experimentalmente la adquisición de la competencia se puede evaluar exponiendo, a diferentes tiempos (horas a días), los explantes a un medio de cultivo adicionado con reguladores del crecimiento y subcultivandolo a medio libre de reguladores del crecimiento.

Este trabajo describe los protocolos de propagación para las especies estudiadas determinando los tiempos de mayor adquisición de competencia para la mayor formación de brotes y/o PLBs a partir del cultivo *in vitro* de protocormos de *Stanhopea tigrina, Laelia anceps, Epidendrum veroscriptum* y *Cattleya* x Esbetts, orquídeas que están bajo alguna categoría de protección o que poseen un alto valor ornamental; hasta donde sabemos no se han realizado ensayos sobre la adquisición de competencia en la familia *Orchidaceae*; por lo cual este primer esfuerzo puede ser encaminado a la producción de un gran número de individuos en un menor tiempo y con menos insumos, que sean la base para cubrir la demanda comercial de que son objeto y contribuir de esta manera a disminuir el saqueo y comercio ilegal de individuos silvestres.

Materiales y métodos

MATERIAL BIOLÓGICO EMPLEADO. Se utilizaron protocormos de tres especies de orquídeas (*Stanhopea tigrina, Laelia anceps, Epidendrum veroscriptum*) y un híbrido (*Cattleya* x Esbetts) provenientes de semillas previamente germinadas en medio basal Murashige y Skoog (MS) (Murashige & Skoog1962). Las cápsulas fueron colectadas a partir de adultos

sanos pertenecientes a la colección del Jardín Botánico Clavijero del Instituto de Ecología, A.C. Se emplearon protocormos de 4 mm de longitud en promedio y que presentaban un estadio de germinación caracterizado por una coloración verde, así como la presencia del primer primordio de hoja y rizoides.

FASE DE INDUCCIÓN. Los protocormos fueron sembrados bajo condiciones asépticas en medio de cultivo MS enriquecido con 100 mg l^{-1} de myo-inositol, 2 mg l^{-1} de glicina y 30 g l^{-1} de sacarosa y adicionado con diferentes concentraciones de N^6-benciladenina (BA) (0, 1, 3, y 5 mg/l) en combinación de ácido 2,4-diclorofenoxiacético (2,4-D) (0 y 0.5 mg/l). El medio fue ajustado a un pH de 5±0.1 con hidróxido de sodio (NaOH) 1N y/o ácido clorhídrico (HCl) 1N, previo a la adición de 8 g/l de agar. El medio de cultivo fue vertido en frascos de vidrio de 120 ml de capacidad y conteniendo 25 ml de medio. Posteriormente se esterilizaron en autoclave durante 15 minutos a una presión de 15 lbs/psi y una temperatura de 120° C.

Se sembraron 20 protocormos por tratamiento colocando 10 explantes por frasco. Para determinar con exactitud el periodo de mayor competencia de los explantes con respecto a los diferentes tratamientos de inducción, se llevó a cabo la siembra y subcultivo en 7 tiempos diferentes: 4, 8, 12, 16, 20, 24 y 28 días, con un total de 1,120 explantes utilizados por especie.

Los cultivos fueron incubados para su desarrollo en un cuarto de crecimiento a una temperatura de 25±2°C, con fotoperiodo de 16 h luz y una densidad de flujo fotónico de 50 μMol m^{-2} s^{-1}.

FASE DE EXPRESIÓN Y DESARROLLO. Después del periodo de inducción, los protocormos fueron subcultivados a medio MS sin reguladores del crecimiento (Medio basal) para evaluar las respuestas morfogenéticas, posteriormente se realizó un nuevo subcultivo después de mes y medio, al mismo medio basal.
Quincenalmente se registraron por explante la talla de cada individuo, el tipo de respuesta morfogenética que presentaron: número de brotes por explante y/o número de cuerpos semejantes a protocormos (PLBs por sus siglas en inglés).

Los brotes obtenidos a partir de los protocormos de las 4 especies, *Stanhopea tigrina, Laelia anceps, Epidendrum veroscriptum* y *Cattleya* x esbetts se individualizaron después de cuatro meses de iniciado el experimento. Los PLBs obtenidos se contabilizaron.

ANÁLISIS ESTADÍSTICO. Los datos obtenidos: número de brotes y PLBs por explante obtenidos a partir de los protocormos cultivados en medio de inducción por diferentes períodos fueron analizados con la ayuda del programa "statistica ver. 2000", mediante un análisis de la varianza de una vía (ANOVA) y los tratamientos fueron discriminados mediante la prueba de rango múltiple de Tukey a $p \leq 0.05$.

Resultados

FORMACIÓN DE PLBS A PARTIR DE PROTOCORMOS DE *S. TIGRINA*. La formación de PLBs fue bastante heterogénea; sólo en algunos explantes fue posible observar PLBs a los 15 días de haber transferido los explantes a medio basal. La base de los protocormos se hinchó adquiriendo una apariencia rugosa debido a la presencia de varias estructuras nodulares, las cuales fueron hinchándose e incrementando en tamaño hasta formar estructuras esféricas de unos 4 mm de diámetro en promedio, con un color verde claro, posteriormente cada PLB comenzó a desarrollar primordios foliares y pelos radiculares en su base, este desarrollo se llevó a cabo a lo largo de 4 meses de iniciado el cultivo. Cada PLB se consolidó en una unidad que se desprendía fácilmente de la masa de PLBs y continuaba con su desarrollo, es decir, crecía y formaba una plántula de alrededor de 0.5-1 cm de talla con una o dos hojas.

No se pudo establecer diferencia significativa entre los tratamientos para la formación de PLBs por explante en *S. tigrina*; sin embargo el tratamiento que indujo la mayor formación de PLBs por explante fue el medio adicionado con 1 y 5 mg/l de BA y con un período de inducción de 24 días, formando en promedio 8 y 7.8 PLBs por explante en cada caso (Fig. 1); por otro lado, hubo varios tratamientos donde la formación de respuestas morfogenéticas fue nula o muy baja (Tabla 1).

FORMACIÓN DE BROTES A PARTIR DE PROTOCORMOS DE *S. TIGRINA*. La consolidación y desarrollo de los brotes producidos a partir de protocormos cultivados en medio de inducción durante distintos períodos, se logró después de transferir los explantes a medio basal, y al ser subcultivados nuevamente a los 45 días al mismo medio, donde el número de brotes se incrementó ligeramente.

Al analizar el conjunto de los tratamientos (concentración de los reguladores del crecimiento y tiempos de inducción) después de 4 meses de cultivo, se pudo establecer diferencia significativa entre los diferentes trata-

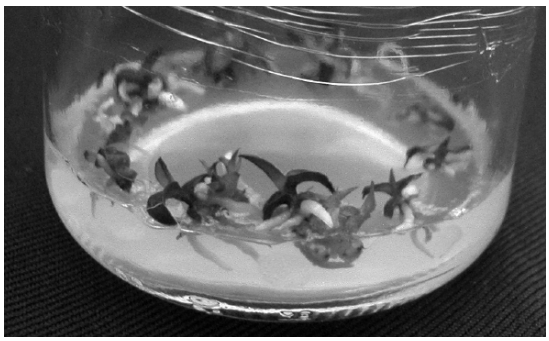

FIGURA 1. Formación de PLBs en protocormos de *Stanhopea tigrina* cultivados con 1 mg/l de BA y con un período de inducción de 24 días.

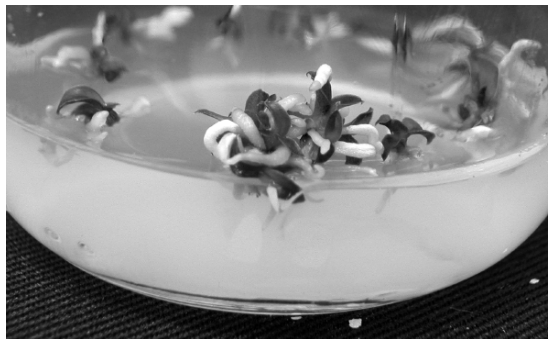

FIGURA 2. Brotación múltiple en protocormos de *Stanhopea tigrina* cultivados en medio adicionado con 5 mg/l de BA y un tiempo de inducción de 24 días.

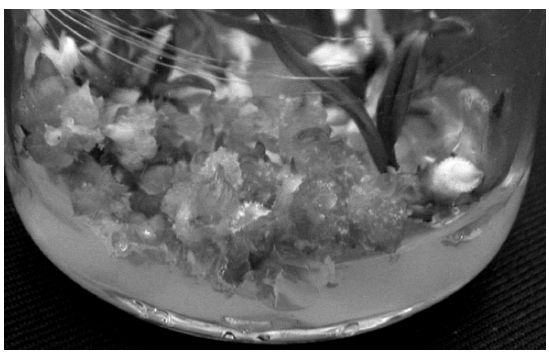

FIGURA 3. Formación de múltiples PLBs a partir de cultivo de protocormos de *Laelia anceps* en medio MS adicionado con diferentes concentraciones de BA y 2,4-D con un tiempo de inducción de 20 días.

FIGURA 4. Brotación múltiple a partir de cultivo de protocormos de *Laelia anceps* en medio MS adicionado con 1 mg/l de BA y 0.5 mg/l de 2,4-D con 4 días de inducción.

mientos ensayados ($p \leq 0.05$). Los tratamientos en los que se logró inducir la mayor formación de brotes por explante a partir del cultivo de protocormos de *S. tigrina*, fueron tres: 1) medio adicionado con 5 mg/l de BA y un tiempo de inducción de 24 días, en el cual se formaron 6.7 brotes por explante (Fig. 2), 2) medio adicionado con 5 mg/l de BA en combinación de 0.5 mg/l de 2,4-D y un tiempo de inducción de 8 días, donde se obtuvo un promedio de 6.4 PLBs; y 3) medio adicionado con 5 mg/l de BA y 0.5 mg/l de 2,4-D, en un período de inducción de 24 días, donde se obtuvo la mayor formación de brotes por explante (8.7) (tabla 2).

FORMACIÓN DE PLBS A PARTIR DE PROTOCORMOS DE *LAELIA ANCEPS* . La formación de PLBs por explante fue muy heterogénea, sin embargo, los resultados en la tabla 3 muestran que la mayor formación de PLBs se dio de manera general en los explantes cultivados en medio de inducción por 20 días para la mayoría de las concentraciones de reguladores empleadas. Cuando los explantes se subcultivaron a medio basal, los PLBs incrementaron ligeramente su tamaño, en algunos los PLBs sufrieron hiperhidratación. Cuando los PLBs alcanzaron 4 mm de diámetro, algunos comenzaron a formar primordios foliares y pelos radiculares, pero después de 4 meses de cultivo muy pocos llegaron a consolidarse como plántulas (Fig. 3).

Al realizar el análisis estadístico para determinar la influencia de los períodos de inducción y las diferentes concentraciones de reguladores empleadas sobre los explantes para la formación de PLBs se pudo establecer una diferencia significativa ($p = 0.000000$). La adquisición de competencia para la mayor formación de PLBs por explante se obtuvo a los 20 días de inducción en dos tratamientos: 1) medio adicionado con 3 mg/l de BA, formando 50.6 PLBs por explante; 2) medio adicionado con 5 mg/l de BA, en el cual se formaron 56.3 PLBs (Tabla 3).

TABLA 1. Promedio de PLBs formados por protocormo de *Stanhopea tigrina* cultivados en medio MS adicionado con diferentes concentraciones de BA y 2,4-D y en diferentes períodos de inducción.

Reguladores de crecimiento (mg/l)		PROMEDIOS FINALES DE PLBs POR PROTOCORMO ± ERROR ESTANDAR							
		Períodos de inducción							PLBs TOTALES
BA	2,4-D	4 días	8 días	12 días	16 días	20 días	24 días	28 días	
0	0	0.05 ± 0.05	0	0	0	0.05 ± 0.05	0	1.1 ± 1.1	24
1	0	0	0.2 ± 0.2	0.5 ± 0.29	1.6 ± 0.73	0.4 ± 0.45	0.1 ± 0.1	**8 ± 7**	218
3	0	0	0	0.3 ± 0.14	1.3 ± 0.81	3.6 ± 3.6	0.8 ± 0.55	0.6 ± 0.48	135
5	0	1.7 ± 0.88	0.05 ± 0.05	0.2 ± 0.2	3.6 ± 2.6	4 ± 2.4	**7.2 ± 2.1**	**7.8 ± 6.5**	**495**
0	0.5	0.1 ± 0.1	0	0	0	0.05 ± 0.05	0	0	3
1	0.5	0.1 ± 0.1	0	0	0	1.8 ± 1.5	3 ± 2	0.4 ± 0.45	107
3	0.5	0.3 ± 0.35	0	2.2 ± 2.25	0	0.8 ± 0.65	0.3 ± 0.3	5.2 ± 4.4	179
5	0.5	0	1.7 ± 1.3	0.2 ± 0.2	1.6 ± 0.96	5.6 ± 2.1	6.9 ± 5.8	0.3 ± 0.26	329

Resultados obtenidos después de 4 meses de cultivo *in vitro*. Números en negritas indican los valores más altos obtenidos.

TABLA 2. Promedio de brotes por explante obtenidos mediante cultivo *in* vitro de protocormos de *Stanhopea tigrina* en medio de cultivo MS, adicionado con diferentes concentraciones de BA y 2,4-D y en diferentes períodos de inducción.

Reguladores de crecimiento (mg/l)		PROMEDIOS FINALES DE PLBs POR PROTOCORMO ± ERROR ESTANDAR							
		Períodos de inducción							PLBs TOTALES
BA	2,4-D	4 días	8 días	12 días	16 días	20 días	24 días	28 días	
0	0	2.6 ± 0.49^{abcde}	1.7 ± 0.19^{ab}	1.2 ± 0.25^{ab}	1.3 ± 0.16^{ab}	1.6 ± 0.16^{abc}	2.7 ± 0.6^{abcde}	1.6 ± 0.26^{abc}	257
1	0	2 ± 0.28^{abcd}	1.3 ± 0.2^{ab}	2 ± 0.45^{abcd}	2.7 ± 0.79^{abcde}	1.4 ± 0.15^{ab}	1.7 ± 0.3^{abc}	2.2 ± 0.44^{abcde}	268
3	0	1.3 ± 0.13^{ab}	1.2 ± 0.15^{a}	1.8 ± 0.3^{abc}	2.7 ± 0.44^{abcde}	3.2 ± 0.78^{abcdef}	2.8 ± 0.71^{abcde}	1.3 ± 0.1^{ab}	289
5	0	1.5 ± 0.36^{ab}	1.4 ± 0.16^{ab}	2.1 ± 0.40^{abcd}	3.7 ± 1.5^{cdefg}	4.2 ± 1.1^{defgh}	$\mathbf{6.7 \pm 1.8^{ij}}$	2.5 ± 0.67^{abcde}	448
0	0.5	2 ± 0.36^{abcd}	1.3 ± 0.1^{ab}	1.6 ± 0.2^{abc}	1.3 ± 0.12^{ab}	2.2 ± 0.46^{abcde}	1.7 ± 0.27^{abc}	1.7 ± 0.16^{abc}	241
1	0.5	2.4 ± 0.55^{abcde}	1.2 ± 0.17^{a}	1.8 ± 0.22^{abc}	1.6 ± 0.18^{abc}	3.4 ± 0.7^{abcdef}	3.3 ± 0.98^{abcdef}	2 ± 0.45^{abcd}	317
3	0.5	1.9 ± 0.21^{abc}	2.2 ± 0.27^{abcd}	2.2 ± 0.36^{abcde}	1.8 ± 0.29^{abc}	2 ± 0.38^{abcd}	5.9 ± 1.3^{ghi}	3 ± 1.1^{abcde}	385
5	0.5	2.7 ± 0.44^{abcde}	$\mathbf{6.4 \pm 3.1^{hi}}$	4.5 ± 1.1^{efghi}	3.5 ± 0.97^{bcdef}	5.4 ± 1.6^{fghi}	$\mathbf{8.7 \pm 2^{j}}$	2.3 ± 0.45^{abcde}	**673**

Resultados obtenidos después de 4 meses de cultivo.

TABLA 3. Promedio de PLBs obtenidos a partir del cultivo *in* vitro de protocormos de *Laelia anceps* en medio de cultivo MS, adicionado con diferentes concentraciones de BA y 2,4-D, en diferentes periodos de inducción.

Reguladores de crecimiento (mg/l)		PROMEDIOS FINALES DE PLBs POR PROTOCORMO ± ERROR ESTANDAR							
		Períodos de inducción							PLBs TOTALES
BA	2,4-D	4 días	8 días	12 días	16 días	20 días	24 días	28 días	
0	0	23.2 ± 14.9^{bcdefghij}	1.1 ± 1.1^{ab}	26.5 ± 14.5^{efghij}	0^{a}	14.2 ± 5.7^{abcdefghi}	3.9 ± 2.3^{abcd}	0^{a}	1379
1	0	25.4 ± 14^{cdefghij}	0.6 ± 0.4^{a}	30.4 ± 23^{ghijk}	0.7 ± 0.4^{a}	33.3 ± 17.2^{hijk}	0^{a}	5.6 ± 3.7^{abcde}	1922
3	0	19 ± 10.6^{abcdefghij}	1.2 ± 0.7^{ab}	3 ± 2^{ab}	12.4 ± 5.8^{abcdefghi}	**50.6 ± 10.9^{kl}**	0.8 ± 0.5^{a}	7.8 ± 3.3^{abcdef}	1900
5	0	8.2 ± 4.7^{abcdefg}	2.3 ± 0.8^{ab}	0.8 ±0.8^{ab}	6.0 ± 2.4^{abcde}	**56.3 ± 8.7^{l}**	5.2 ± 2.5^{abcde}	4.6 ± 2.9^{abcde}	1672
0	0.5	7.8 ± 3.6^{abcdef}	32.8 ± 15.9^{hijk}	21.9 ± 12.9^{abcdefghij}	0^{a}	0^{a}	13.4 ± 7.3^{abcdefghi}	0^{a}	1521
1	0.5	11.3 ± 4.4^{abcdefgh}	0^{a}	10.1 ± 6^{abcdefg}	2.3 ± 1.5^{ab}	34.4 ± 18.3^{ijkl}	2.9 ± 2.2^{ab}	5.4 ± 5.4^{abcde}	1331
3	0.5	4 ± 1.9^{abcd}	2.5 ± 1.6^{ab}	29.8 ± 13.2^{fghijk}	12.5 ± 5.3^{abcdefghi}	1.2 ± 0.8^{ab}	3.8 ± 2.9^{abcd}	3.2 ± 2.2^{abc}	1143
5	0.5	25.6 ±12.2^{defghij}	41.3 ± 13.4^{jkl}	37.7 ± 12.2^{jkl}	14.1 ± 6.6^{abcdefghi}	6.1 ± 2.7^{abcde}	6.2 ± 3.3^{abcde}	6.3 ± 2.9^{abcde}	**2748**

Resultados obtenidos después de 4 meses de cultivo.

TABLA 4. Promedio final de brotes por explante, obtenidos mediante cultivo *in* vitro de protocormos de *Laelia anceps* en medio de cultivo MS, adicionado con diferentes concentraciones de BA y 2,4-D, en diferentes periodos de inducción.

Reguladores de crecimiento (mg/l)		PROMEDIOS FINALES DE PLBs POR PROTOCORMO ± ERROR ESTANDAR							
		Períodos de inducción							PLBs TOTALES
BA	2,4-D	4 días	8 días	12 días	16 días	20 días	24 días	28 días	
0	0	**26.5 ± 12.2^{k}**	6.5 ± 2.8^{abcd}	3.4 ± 0.5^{ab}	3 ± 0.5^{a}	11.5 ± 1.7^{abcdefg}	6 ± 1.8^{abcd}	3.6 ± 1.1^{ab}	1212
1	0	21.8 ± 7.3^{hijk}	5 ± 1.4^{ab}	8.1 ± 1.8^{abcde}	5.1 ± 0.9^{ab}	17.7 ± 4^{efghijk}	6.1 ± 1.4^{aabcd}	8.8 ± 2.9^{abcde}	1457
3	0	12.4 ± 3.8^{abcdefghi}	6.2 ± 0.9^{abcd}	15.1 ± 1.8^{cdefghij}	8.6 ± 2.4^{abcde}	22.3 ± 4.2^{ijk}	15.8 ± 3^{defghij}	10.3 ± 2.6^{abcde}	**1816**
5	0	6.6 ± 1.8^{abcd}	5.6 ± 0.9^{abc}	10.1 ± 2.9^{abcde}	4.1 ± 1.5^{ab}	21.8 ± 2.9^{hijk}	7.8 ± 2^{abcde}	11.9 ± 3.2^{abcdefghi}	1363
0	0.5	13 ± 3.6^{bcdefghi}	4.9 ± 1.4^{ab}	3 ± 0.6^{a}	4.7 ± 1.9^{ab}	4.1 ± 0.6^{ab}	3.4 ± 0.9^{ab}	3.5 ± 0.9^{ab}	736
1	0.5	**24 ± 9.9^{jk}**	4.2 ± 1.1^{ab}	7.9 ± 1.9^{abcde}	8.5 ± 0.4^{abcde}	20.3 ± 6.1^{fghijk}	4.4 ± 1^{ab}	6.8 ± 2.4^{abcd}	1524
3	0.5	**23.6 ± 10.1^{jk}**	7.5 ± 1.2^{abcd}	6.4 ± 1.5^{abcd}	8.3 ± 1.6^{abcde}	5.7 ± 1.4^{abc}	6.9 ± 1.8^{abcd}	4.9 ± 0.9^{ab}	1268
5	0.5	20.8 ± 6.9^{ghijk}	8 ± 2^{abcde}	4.5 ± 1.1^{ab}	8.3 ± 1.9^{abcde}	10.9 ± 3^{abcdef}	7.7 ± 1.2^{abcd}	7.5 ± 1.8^{abcd}	1356

Resultados obtenidos después de 4 meses de cultivo.

FIGURA 5. Formación de múltiples PLBs en protocormos de *Epidendrum veroscriptum* cultivados en medio MS con 1 mg/l de BA en y 0.5 mg/l de 2,4-D con un tiempo de inducción de 12 días.

FIGURA 6. Brotación múltiple a partir de cultivo de protocormos de *Epidendrum veroscriptum* cultivados en medio MS adicionado con 3 mg/l de BA y 0.5 mg/l de 2,4-D con un tiempo de inducción de 20 días.

FORMACIÓN DE BROTES A PARTIR DE PROTOCORMOS DE *L. ANCEPS.* Para los explantes de *L. anceps* la formación de brotes fue heterogénea. La consolidación y desarrollo de los brotes producidos fue manifestándose después de transferirlos a medio basal, los brotes fueron desarrollando primordios foliares y raíz hasta convertirse en plántulas completas. Cuando fueron subcultivados después de 45 días a medio basal, el número de brotes se incrementó considerablemente, llegando en algunos casos a duplicar o incluso a triplicar la cantidad de brotes producidos por explante.

Al analizar el efecto del tiempo de inducción para la formación de brotes en el conjunto de los tratamientos después de 4 meses de cultivo, se logró establecer diferencia significativa ($p = 0.000000$) entre las concentraciones y tiempos de inducción utilizadas. De acuerdo con la tabla 4 se observa que el mayor período de inducción se obtuvo en: 1) medio sin reguladores del crecimiento, formando un promedio de 26.5 brotes por explante en un tiempo de 4 días de inducción; 2) medio adicionado con 1 mg/l de BA en combinación con 0.5 mg/l de 2,4-D, que formó 24.9 brotes por explante a los 4 días de inducción (Fig. 4), y 3) medio adicionado con 3 mg/l de BA en combinación con 0.5 mg/l de 2,4-D, con un promedio de 23.6 brotes. El promedio más bajo para la formación de brotes por explante fue de 3 en dos períodos de inducción: 1) medio sin reguladores del crecimiento, con un tiempo de inducción de 16 días, y 2) el medio adicionado con 1 mg/l de BA en combinación con 0.5 mg/l de 2,4-D (tabla 4).

Después de 4 meses de cultivo, 19.19% del total de los protocormos de *L. anceps* (1120) germinaron y formaron una plántula, con hojas y raíz, solamente en algunos casos formaron pseudobulbos, con una apariencia vigorosa y una coloración verde oscura en las hojas, el 72.41% presentó respuestas morfogenéticas, como brotes y PLBs. y el 8.39 % del material biológico se perdió debido a necrosis de los protocormos o por contaminación.

FORMACIÓN DE PLBS A PARTIR DE PROTOCORMOS DE E. VEROSCRIPTUM. La formación de PLBs a partir de los protocormos de *E. veroscriptum* cultivados en diferentes concentraciones de reguladores del crecimiento y en diferentes tiempos de inducción, fue de manera general baja. La mayor formación de PLBs por explante se obtuvo con un tiempo de inducción de 12 días y a partir del control y del tratamiento adicionados con 1 mg/l de BA en combinación con 0.5 mg/l de 2,4-D se logró formar 15.3 y 7.2 PLBs por explante en promedio respectivamente (Tabla 5).

Cuando los explantes fueron subcultivados a medio libre de reguladores del crecimiento (MB), la formación y desarrollo de PLBs por explante fue gradual, es decir, los PLBs comenzaron a aparecer como pequeños nódulos, los cuales fueron hinchándose hasta tener una estructura semiesférica, con apariencia rugosa, en todos los casos los PLBs siempre se formaron como estructuras independientes que se desprendían fácilmente del explante original. Cuando los PLBs alcanzaron una talla de 5 mm en promedio, comenzaron a desarrollar primordio foliar y rizoides (Fig. 5).

FORMACIÓN DE BROTES A PARTIR DE PROTOCORMOS DE *E. VEROSCRIPTUM.* La formación de brotes a partir de los protocormos no fue evidente sino hasta los protocormos fueron subcultivados a medio basal, quince días después era perceptible la formación de primordios de brote.

TABLA 5. Promedio de PLBs obtenidos a partir del cultivo *in vitro* de protocormos de *Epidendrum veroscriptum* en medio de cultivo MS, adicionado con diferentes concentraciones de BA y 2,4-D, en diferentes periodos de inducción.

Reguladores de crecimiento (mg/l)		PROMEDIOS FINALES DE PLBs POR PROTOCORMO ± ERROR ESTANDAR							
		Períodos de inducción							PLBs TOTALES
BA	2,4-D	4 días	8 días	12 días	16 días	20 días	24 días	28 días	
0	0	0.5 ± 0.4^{abc}	0^{a}	$\mathbf{15.3 \pm 7.8^{f}}$	0^{a}	1.1 ± 0.8^{abc}	2 ± 1.3^{abcd}	0.6 ± 0.3^{abc}	394
1	0	2 ± 1.2^{abcd}	0.7 ± 0.7^{abc}	0.3 ± 0.3^{ab}	0.6 ± 0.4^{abc}	2.7 ± 2^{abcd}	5.3 ± 2.3^{abcde}	3 ± 1.5^{abcd}	296
3	0	1.6 ± 1^{abcd}	0.1 ± 0.1^{ab}	0.2 ± 0.2^{ab}	1.7 ± 1.4^{abcd}	4.3 ± 2.2^{abcde}	2.2 ± 1.4^{abcd}	3.7 ± 2.3^{abcde}	281
5	0	0.4 ± 0.2^{ab}	0^{a}	0^{a}	0^{a}	0.8 ± 0.4^{abc}	0.4 ± 0.4^{ab}	1.1 ± 0.4^{abc}	56
0	0.5	3.7 ± 3.2^{abcde}	0.9 ± 0.7^{ab}	0^{a}	3 ± 1.9^{abcd}	5.7 ± 2.3^{bcde}	1.8 ± 1.2^{abcd}	3.6 ± 1.4^{abcde}	377
1	0.5	2.6 ± 1^{abcd}	1.8 ± 0.7^{abcd}	$\mathbf{7.2 \pm 7.2^{de}}$	6.2 ± 3^{cde}	0.7 ± 0.5^{abc}	0.3 ± 0.2^{ab}	1.7 ± 0.6^{abcd}	413
3	0.5	3.9 ± 3.8^{abcde}	5.7 ± 2.5^{bcde}	0^{a}	1.8 ± 0.9^{abcd}	1.1 ± 0.7^{abc}	5.5 ± 2.4^{abcde}	3.8 ± 1.9^{abcde}	436
5	0.5	4.9 ± 2.2^{abcde}	0.5 ± 0.3^{abc}	0^{a}	8.8 ± 2.8^{e}	0.3 ± 0.3^{ab}	2.3 ± 1.3^{abcd}	2.5 ± 1.1^{abcd}	392

Resultados obtenidos después de 4 meses de cultivo *in vitro*.
Nota: letras diferentes indican diferencia significativa ($p \leq 0.05$).

TABLA 6. Promedio final de brotes por explante, obtenidos mediante cultivo *in* vitro de protocormos de *Epidendrum veroscriptum* en medio de cultivo MS, adicionado con diferentes concentraciones de BA y 2,4-D, en diferentes periodos de inducción.

Reguladores de crecimiento (mg/l)		PROMEDIOS FINALES DE PLBs POR PROTOCORMO ± ERROR ESTANDAR							
		Períodos de inducción							PLBs TOTALES
BA	2,4-D	4 días	8 días	12 días	16 días	20 días	24 días	28 días	
0	0	3.3 ± 1^{abcde}	2.9 ± 1^{abcde}	6.1 ± 1.6^{cdefghij}	2.6 ± 0.9^{abcde}	5.3 ± 1.4^{abcdefghi}	4.1 ± 1.4^{abcdefg}	2.1 ± 0.4^{abc}	531
1	0	4.3 ± 1.6^{abcdefg}	2.3 ± 0.4^{abcde}	3.1 ± 1^{abcde}	2.2 ± 0.5^{abcd}	4 ± 1.3^{abcdefg}	2.9 ± 0.5^{abcde}	4.8 ± 1.9^{abcdefg}	475
3	0	2.4 ± 0.4^{abcde}	3.1 ± 0.4^{abcde}	2.1 ± 0.7^{abc}	4.2 ± 1.2^{abcdefg}	6.2 ± 2.2^{defghij}	2.4 ± 0.5^{abcde}	2.7 ± 0.6^{abcde}	467
5	0	3.8 ± 0.6^{abcdef}	2.1 ± 0.5^{abc}	2.1 ± 0.5^{abc}	1.4 ± 0.1^{a}	3.6 ± 0.8^{abcde}	2.7 ± 0.5^{abcde}	1.3 ± 0.2^{a}	343
0	0.5	5.1 ± 1.6^{abcdefghi}	4.1 ± 0.7^{abcdefg}	3.8 ± 1.6^{abcdefg}	4 ± 1.5^{abcdefg}	3.6 ± 1.3^{abcde}	5.7 ± 1.3^{bcdefghij}	2 ± 0.3^{ab}	569
1	0.5	3 ± 0.7^{abcde}	5.3 ± 2.1^{abcdefghi}	5 ± 1.3^{abcdefg}	$\mathbf{9.1 \pm 2.1^{hij}}$	6.3 ± 1.9^{efghij}	5.1 ± 3.5^{abcdefgh}	4.9 ± 1.3^{abcdefg}	777
3	0.5	5.5 ± 2^{bcdefghi}	7.9 ± 1.8^{ghij}	4.1 ± 0.7^{abcdefg}	$\mathbf{9.6 \pm 2.4^{j}}$	$\mathbf{9.2 \pm 3.7^{ij}}$	5.9 ± 2.6^{bcdefghij}	5.1 ± 1.1^{abcdefghi}	950
5	0.5	4.4 ± 0.9^{abcdefg}	3.6 ± 0.9^{abcde}	3.5 ± 1.1^{abcde}	7.8 ± 2.2^{fghij}	3.3 ± 0.6^{abcde}	4 ± 1^{abcdefg}	5.5 ± 1.2^{bcdefghi}	645

Resultados obtenidos después de 4 meses de cultivo.

FIGURA 7. Formación de múltiples PLBs a partir de cultivo de protocormos de *Cattleya* x esbetts cultivados en medio MS adicionado con 3 mg/l de BA y 0.5 mg/l de 2,4-D por 4 días

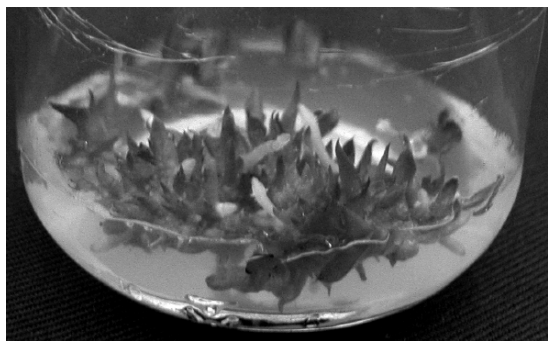

FIGURA 8. Brotación múltiple a partir de cultivo de protocormos de *Cattleya* x esbetts cultivados en medio MS adicionado con 1 mg/l de BA con un período de inducción de 20 días.

Al analizar los datos registrados para la formación de brotes por explante fue posible establecer diferencia significativa entre los diferentes tiempos de inducción y concentraciones de reguladores empleadas ($p=0.001387$). Los datos en la tabla 6 muestran que la adquisición de competencia para la mayor formación de brotes se obtuvo en tres tratamientos: medio adicionado con 3 mg/l de BA y 0.5 mg/l de 2,4-D los cuales, con períodos de 16 días y 20 días de inducción, formaron 9.6 y 9.2 brotes por explante respectivamente y medio de cultivo adicionado con 1 mg/l de BA y 0.5 mg/l de 2,4-D, con un período de inducción de 16 días, formaron 9.1 brotes por explante en promedio (Fig. 6).

Cattleya x Esbetts

FORMACIÓN DE PLBS A PARTIR DEL CULTIVO *IN VITRO* DE PROTOCORMOS DE *C.* X ESBETTS. Al analizar el efecto que tuvieron los períodos de inducción en los explantes para la formación de PLBs entre los tratamientos ensayados, fue posible establecer diferencia significativa ($p = 0.000208$). En la tabla 7 se puede observar que la formación de PLBs fue en general baja, y solamente en algunos tratamientos se obtuvo una gran cantidad de PLBs por explante. La mayor formación de PLBs se obtuvo a partir de los explantes cultivados en medio de inducción por 4 días en dos tratamientos, el adicionado con 3 mg/l de BA y 0.5 mg/l de 2,4-D, los cuales produjeron un promedio de 22.2 PLBs por explante y en el medio adicionado con 1 mg/l de BA y 0.5 mg/l de 2,4-D, en donde los explantes formaron 20.8 PLBs en promedio (Fig. 7). En algunos tratamientos no se obtuvo la formación de PLBs, principalmente con un tiempo de inducción de 12 días (tabla 7).

FORMACION DE BROTES A PARTIR DEL CULTIVO DE PROTOCORMOS DE *CATTLEYA* X ESBETTS. Al analizar el efecto de los períodos de inducción para la formación de brotes por explante, fue posible establecer diferencia significativa ($p\leq0.5$). Los datos en la tabla 8 muestran que la adquisición de competencia para la mayor formación de brotes por explante se obtuvo en tres tratamientos: 1) en medio MS adicionado con 1 mg/l de BA en un período de inducción de 20 días, donde se obtuvo una formación promedio de 18.7 brotes por explante; 2) en medio MS adicionado con 3 mg/l de BA por 16 días, donde se logró una formación de 17.8 brotes por explante en promedio; 3) en el medio adicionado con 3 mg/l de BA y 0.5 mg/l de 2,4-D, con un período de inducción de 28 días, obteniendo un promedio de 16.5 brotes por explante (Fig. 8). Por el otro lado, la menor capacidad regenerativa para la formación de brotes se registró en los explantes cultivados en medio de inducción adicionado con 1 mg/l de BA por 28 días, los cuales formaron alrededor de 0.9 brotes por explante (tabla 8).

Discusión

USO DE PROTOCORMOS EN LA MICROPROPAGACIØÓN.

Los protocormos derivados de semillas de orquídeas fueron una importante fuente de explante para los propósitos de este trabajo, pues se sabe que las semillas son el material de diversidad genética por excelencia que se debe tomar en cuenta cuando se requiere recuperar especies que se encuentren amenazadas, pues garantizan una estabilidad y diversidad genética (Martínez, 1985). Los protocormos pueden ser utilizados lo mismo para incrementar su talla y consolidarlos como plántulas, como es el caso del trabajo de Fonnesbech (1972),

TABLA 7. Promedio de PLBs obtenidos a partir del cultivo *in* vitro de protocormos de *Cattleya* x esbetts en medio de cultivo MS, adicionado con diferentes concentraciones de BA y 2,4-D, en diferentes periodos de inducción.

Reguladores de crecimiento (mg/l)		PROMEDIOS FINALES DE PLBs POR PROTOCORMO ± ERROR ESTANDAR							
		Períodos de inducción							PLBs TOTALES
BA	2,4-D	4 días	8 días	12 días	16 días	20 días	24 días	28 días	
0	0	0.3 ± 0.2^{a}	0^{a}	0.3 ± 0.3^{a}	0.8 ± 0.5^{abc}	0.7 ± 0.6^{abc}	0.3 ± 0.3^{a}	0^{a}	50
1	0	3.7 ± 1.8^{abcd}	0.1 ± 0.1^{a}	1.1 ± 1.1^{abc}	2.6 ± 1.8^{abcd}	5.8 ± 3.2^{abcd}	0^{a}	0.5 ± 0.5^{ab}	280
3	0	2.8 ± 1.5^{abcd}	0^{a}	9.4 ± 2.2^{bcd}	2.4 ± 1^{abcd}	6.7 ± 4^{abcd}	1.8 ± 1^{abcd}	6.5 ± 3.4^{abcd}	594
5	0	0.5 ± 0.5^{ab}	0^{a}	2.8 ± 1.6^{abcd}	0.6 ± 0.5^{ab}	2.8 ± 1.3^{abcd}	3.6 ± 1.9^{abcd}	4.4 ± 3.5^{abcd}	298
0	0.5	2.9 ± 1.7^{abcd}	3.3 ± 2.8^{abcd}	1.6 ± 1.6^{abcd}	0.5 ± 0.5^{ab}	4.5 ± 4.5^{abcd}	0.5 ± 0.5^{ab}	0.7 ± 0.7^{ab}	281
1	0.5	**20.8 ± 15.3^{e}**	1.8 ± 1.3^{abcd}	6.2 ± 2.9^{abcd}	3.6 ± 1.5^{abcd}	0^{a}	2.1 ± 1.4^{abcd}	1.9 ± 1.4^{abcd}	733
3	0.5	**22.2 ± 12.2^{e}**	1.5 ± 1^{abcd}	0^{a}	6.2 ± 3.2^{abcd}	1.4 ± 0.9^{abc}	2.4 ± 1.9^{abcd}	**10.5 ± 2.8^{d}**	889
5	0.5	3.2 ± 1.8^{abcd}	7.2 ± 3.1^{abcd}	1.1 ± 0.6^{abc}	2.8 ± 1.1^{abcd}	7.1 ± 4.4^{abcd}	9.7 ± 3.4^{cd}	5.2 ± 2^{abcd}	730

Resultados obtenidos después de 4 meses de cultivo *in vitro*

Nota: letras diferentes indican diferencia significativa ($p \leq 0.05$)

TABLA 8. Promedio final de brotes por explante, obtenidos mediante cultivo *in* vitro de protocormos de *Cattleya* x Esbetts en medio de cultivo MS, adicionado con diferentes concentraciones de BA y 2,4-D, en diferentes periodos de inducción..

Reguladores de crecimiento (mg/l)		PROMEDIOS FINALES DE PLBs POR PROTOCORMO ± ERROR ESTANDAR							
		Períodos de inducción							PLBs TOTALES
BA	2,4-D	4 días	8 días	12 días	16 días	20 días	24 días	28 días	
0	0	3.5 ± 0.6^{abcdefg}	5.9 ± 0.6^{abcdefghij}	4.2 ± 1.7^{abcdefg}	$6.5 \pm 1.9^{abcdefghijk}$	9 ± 2.3^{cdefghijk}	1.6 ± 0.2^{ab}	1.3 ± 0.2^{ab}	644
1	0	3.5 ± 0.5^{abcdef}	2.6 ± 1.4^{abcde}	4.6 ± 2^{abcdefgh}	8.7 ± 2.5^{cdefghijk}	**18.7 ± 5.4^{n}**	8.7 ± 2.5^{cdefghijk}	0.9 ± 0.06^{a}	947
3	0	4.3 ± 1.7^{abcdefgh}	5.2 ± 1.4^{abcdefgh}	9.1 ± 1.6^{defghijk}	**17.8 ± 4.6^{mn}**	$6.4 \pm 1.9^{abcdefghijk}$	2.7 ± 1^{abcde}	5 ± 1^{abcdefgh}	1012
5	0	3 ± 0.7^{abcdef}	4.7 ± 1.7^{abcdefgh}	4.1 ± 1.3^{abcdefg}	12.9 ± 3.1^{jklmn}	3.1 ± 0.9^{abcdefg}	9.6 ± 2.1^{efghijkl}	8.9 ± 3.7^{cdefghijk}	927
0	0.5	5 ± 1^{abcdefgh}	5.6 ± 2^{abcdefghi}	3.1 ± 0.7^{abcdefg}	1.5 ± 0.2^{ab}	5.4 ± 1.7^{abcdefgh}	2.5 ± 0.6^{abcde}	2 ± 0.4^{abc}	505
1	0.5	9.4 ± 2.8^{defghijk}	13.1 ± 9.7^{klmn}	11.3 ± 2.4^{hijklm}	4.9 ± 1.4^{abcdefgh}	$6.9 \pm 1.9^{abcdefghijk}$	2.4 ± 0.7^{abcd}	4.6 ± 1.3^{abcdefgh}	1054
3	0.5	9.4 ± 2.8^{defghijkl}	3.4 ± 1.2^{abcdefg}	12.6 ± 4.5^{ijklmn}	9.2 ± 3.3^{defghijk}	$7.4 \pm 2.8^{abcdefghijk}$	2.4 ± 1.1^{abcd}	**16.5 ± 3.4^{lmn}**	1032
5	0.5	4.7 ± 1.5^{abcdefgh}	8.3 ± 2^{bcdefghijk}	12.6 ± 2.4^{ijklmn}	10 ± 2.7^{fghijkl}	12.6 ± 1.8^{ijklmn}	10.1 ± 2.9^{ghijkl}	5.1 ± 1.3^{abcdefgh}	1272

Resultados obtenidos después de 4 meses de cultivo *in vitro*

Nota: letras diferentes indican diferencia significativa ($p \leq 0.05$)

o bien para que sigan una ruta específica de desarrollo (Ramírez, 1990; Park *et al*., 2002; Faria *et al*., 2004; Ket *et al*., 2004 y Malabadi *et al*., 2005). Aunque se utilizó la misma técnica de cultivo para las cuatro especies estudiadas (cultivo de protocormos en medio de inducción MS y posteriormente medio basal) los resultados fueron diferenciales, ya que las concentraciones de los reguladores de crecimiento y los tiempos de inducción empleados fueron diferentes y se obtuvieron respuestas morfogenéticas distintas.

La principal respuesta a partir del cultivo de protocormos de *L. anceps*, *E. veroscriptum* y *Cattleya* x esbetts fue la formación de PLBs, mientras que en *Stanhopea tigrina* fue la formación de brotes. Baltazar (2004) reporta en su trabajo que la principal respuesta al cultivo *in vitro* de protocormos enteros en medio KC y MS con combinaciones factoriales de reguladores del crecimiento fueron brotes adventicios, mientras que a partir de mitades de protocormos la principal respuesta que obtuvo fue la formación de PLBs. Shyamal y Pinaki, (2004) obtuvieron regeneración de brotes a partir del cultivo de semillas de *Vanda teres* (Roxb.) Lindl. en medio Vacin and Went adicionado con 1.0 mg/l BAP y 0.5 mg/l ANA. Por su parte, Lu (2004) consiguió una alta brotación a partir del cultivo de protocormos de *Pleione formosana* Hayata en medio MS al 50% adicionado con 5 mg/l de 2,4-D y 0.5 mg/l de Tidiazurón. Además, Cacalvante *et a*l., (2001) promovieron la expresión de respuestas morfogenéticas vía organogénesis indirecta a partir de protocormos cultivados en medio MS adicionado con 0.5 y 1 mg/l de BAP en *Góngora quinquenervis*, Por último, Bhadra y Hossain (2003) también obtuvieron diferentes respuestas morfogenéticas a partir del cultivo de protocormos de *Geodorum densiflorum* (Lam.) Schltr. en medio MS adicionado con diferentes combinaciones de reguladores del crecimiento.

EFECTO DE LOS TRATAMIENTOS EN LA ADQUISICIÓN DE COMPETENCIA PARA LA FORMACION DE PLBS.

En todas las especies estudiadas los PLBs fueron inducidos directamente a partir de los explantes originales de cada una de las especies y la respuesta a la inducción fue heterogénea. La mayor formación de PLBs se presentó principalmente en *L. anceps* con 20 días de inducción y con altas concentraciones de BA (3 y 5 mg/l) y en ausencia de 2,4-D, donde se indujo la formación de 50 a 56 PLBs por explante en promedio, en *Cattleya* x esbetts, cuyos explantes cultivados en medio con BA (1 y 3 mg/l) en presencia de 2,4-D por 4 días, los cuales promovieron la formación de 20 a 22 PLBs por explante al final del experimento. En el caso de *E. veroscriptum*, la capacidad regenerativa más alta para la formación de PLBs se obtuvo con 12 días de inducción, tanto en el medio sin reguladores del crecimiento como el medio con una baja concentración de BA (1 mg/l) y 2,4-D, mientras que la concentración más alta de BA en ausencia de 2,4-D inhibió la formación de PLBs en los explantes cultivados. Por su parte, los explantes de *S. tigrina* cultivados en medio de inducción por 28 días tuvieron una fuerte influencia de BA en (1 y 3 mg/l) sin 2,4-D, así como en altas concentraciones de BA en presencia de 2,4-D, las cuales promovieron la mayor formación de PLBs, mientras que bajas concentraciones de BA con 2,4-D inhibieron fuertemente la producción de PLBs, estos resultados contrastan con lo reportado por Mata y Salazar (2003), pues ellos obtuvieron la mayor formación de PLBs en protocormos de *Cuitlauzinia pendula* que fueron cultivadas en medio MS sin reguladores de crecimiento y en medio adicionado con 0.5 mg/l BA por 4 meses. Lo anterior demuestra que los requerimientos para la inducción (micropropagación) de las diferentes especies de orquídeas, es decir, medio de cultivo, concentración de reguladores del crecimiento y tiempo de inducción, deben ser determinadas experimentalmente.

EFECTO DE LOS TRATAMIENTOS EN LA ADQUISICIÓN DE COMPETENCIA PARA LA FORMACION DE BROTES.

Para *L. anceps* la mayor formación de brotes, 26.5 brotes por explante, se obtuvo con 4 días de inducción en medio MS sin reguladores de crecimiento. Ha sido demostrado que algunas veces, no siempre se requiere de los reguladores del crecimiento para obtener la respuesta morfogenética adecuada, ya que puede lograrse por medio de factores físicos o químicos que afectan la movilización, producción o inhabilitación de los reguladores del crecimiento en el explante; los niveles endógenos de los reguladores son influenciados por la duración, calidad e intensidad de la luz y por factores químicos ambientales, tales como macro y micronutrientes, además, la activación o inactivación de las rutas metabólicas por la biosíntesis de aminoácidos aromáticos y la respiración pueden cambiar los niveles endógenos hormonales en el explante (Bonga y Aderkas, 1992).

En *Cattleya* x Esbetts la presencia de BA fue deter-

minante para la mayor inducción de brotes por explante, logrando inducir la formación hasta 18.7 brotes por explante en aquellos explantes cultivados únicamente con 1 mg/l de BA, este resultado concuerda con lo reportado por Arditti (1992), quien afirma que después de varios estudios realizados en *Cattleya*, las combinaciones óptimas para la formación de brotes para este género fue 1 mg/l de BA y 0.5 mg/l de ANA. Es frecuente encontrar reportes en la propagación *in vitro* de orquídeas el uso de bajas concentraciones de citocininas con nulas y muy bajas concentraciones de auxinas, Baltazar (2004) reporta que las mejores respuestas morfogenéticas en el cultivo de protocormos enteros de *Oncidium tigrinum* en medio de cultivo MS con una concentración similar (0.1 mg/l de ANA y 1 mg/l de BA), mientras que Krapiec *et al*., (2003) obtuvieron una alta brotación a partir de protocormos cultivados en medio B5 con diferentes combinaciones de BA y AIB. Por su parte, George y Ravishankor (1997) mencionan en su trabajo con *Vanilla plannifolia* que obtuvieron respuestas morfogenéticas en medio adicionado con 2 mg/l de BA y 1 mg/l de ANA.

En *S. tigrina* y *E. veroscriptum* se logró la inducción de brotes entre los 16 y los 24 días con altas concentraciones de BA en presencia de 2,4-D, obteniendo entre 8 y 9 brotes por explante. Además, se ha podido constatar de manera general, que la presencia de 2,4-D en el medio inhibió la multiplicación de brotes, lo cual no afectó su crecimiento, alcanzando en algunos casos las tallas más altas para cada especie, lo cual es similar nuevamente con lo que reporta Arditti (1992), quien determinó que muy bajas concentraciones (0.1 mg/l) de ANA o 2,4-D promueven el crecimiento de los explantes.

Conclusiones

Es muy común encontrar en la literatura referente a la propagación de orquídeas y otras especies tiempos de inducción que van de 30 a 60 días y son muy pocos los que mencionan estudios específicos para determinar los tiempos de adquisición de competencia, la gran mayoría de trabajos de este tipo se han realizado con coníferas y no hemos encontrado ninguno con orquídeas, por lo que este trabajo puede considerarse pionero en la investigación acerca de la adquisición de competencia en esta familia. El poder determinar con una mayor precisión el momento en que el explante a cultivar adquiere competencia para la formación de brotes y/o PLBs no sólo se logrará inducir la mayor cantidad de regenerantes por explante, sino que también se podrán reducir los tiempo y costos de producción, teniendo como consecuencia, material suficiente que pueda ser usado ya sea para realizar otro tipo de estudios (anatómico, fisiológico, genético, ecológicos etc.) o plantas de las especie silvestres que puedan ser usadas para satisfacer el comercio legal con lo que se estará contribuyendo a disminuir la presión de colecta, en muchos casos ilegal, de sus poblaciones silvestres, con le que se estará incidiendo directamente con la conservación y uso sustentable de este valioso recurso natural. En caso de los híbridos con alto valor ornamental y de las mismas especies el poder reducir los tiempos y costos de producción y contar con plantas sanas y vigorosas será posible ofrecerlas al consumidor de manera controlada y a precios altamente competitivos y accesibles.

Literatura citada

Adelberg, J., R. Pollock, N. Rajapakse & R. Young. 1998. Micropropagation, decontamination, transcontinental shipping and hydroponic growth of *Cattleya* while sealed in semipermeable membrane vessels. Scientia Horticulturae 73:23–35.

Arditti, J., E.A. Ball & M.E. Churchill. 1971. Propagación clonal de orquídeas utilizando ápices de hojas. Orquídea (Mexico) 2: 290–300.

Arditti, J. 1992. Fundamentals of orchid biology. John Wiley and Sons. U. S. A. 691 p.

Ávila, I. & K. Oyama. 2002. Manejo sustentable de *Laelia speciosa* (Orchidaceae). Biodiversitas. 7(43):9-12.

Azcón-Bieto, J. & M.Talón. 2001. Capítulo 18. Fundamentos de fisiología vegetal. 2da reimpresión. Mc Graw-Hill. Interamericana de España. Edicions Universitat de Barcelona. España. 522 p.

Baltazar, R. 2004. Micropropagación de *Oncidium tigrinum* Llave y Lex. (Orchidaceae) a partir de protocormos. Tesis de licenciatura (Biología). Universidad Veracruzana. Xalapa, Ver. 124 p.

Bechtel, H., P. Cribb & E. Launert. 1981. The manual of cultivated orchid species. The MIT Press. Cambridge. 443 p.

Bhadra, S. & M. Hossain. 2003. *In vitro* Germination and Micropropagation of *Geodorum densiflorum* (Lam.) Schltr., an Endangered Orchid Species. Pl. Tissue Cult. 13(2): 165-171.

Bonga, J. & P. Aderkas. 1992. *In vitro* culture of trees. Kluwer Academic Publishers. The Netherlands. 209 p.

Capesius I. & Y. Meyer. 1977. Isolation of nuclei from protoplasts of orchids. Cytobiol. 15: 485–490.

Cavalcante, P., L. Willadino, G. Dias & V. Tenorio. 2001. Pesq. Agropec. Bras., Brasil. 36(10): 1319-1324.

Champagnat, M., G.Morel & B. Mounetou. 1970. La multiplication végétative des *Cattleya é* partir de jeunes feuilles cultivées in vitro. Ann. Sc. Nat. Bot. Biol. Vég. (Sér. 12) 11: 97–114.

Churchill, M.-E., E.A. Ball & J. Arditti. 1972. Tissue culture of orchids. II. Methods for root tips. Amer. Orchid Soc. Bull. 41: 726–730.

Churchill, M.-E., E.A. Ball & J. Arditti. 1973. Tissue culture of orchids. I. Methods for leaf tips. New Phytol. 72:161–166.

Dhaliwal, H., N. Remesar-Forther, E. Yeung & T. Thorpe. 2003. Competence, determination, and meristemoid plasticity in tobacco organogenesis *in vitro*. Canadian Journal of Botany. Ottawa. Vol. 81, lss. 6; p. 611.

Dixon, K.W., S.P.Kell & P.J. Cribb (eds.). 2003. Orchid Conservation. Natural History Publications (Borneo), Kota Kinabalu, Sabah. Pp. 259-288.

Dressler, R. L. 1993. Phylogeny and classification of the orchid Family. Dioscorides Press. Portland, Oregon. 313 p.

Ellis, D. & D.Bildebarck. 1989. Temporal competence of embryonic *Pinus ponderosa* cotyledons to form multiple buds *in vitro*. Amer. J. Bot. 76(3): 348-355.

Espejo, A., J. García, A. López, R. Jiménez & L. Sánchez. 2002. Orquídeas del estado de Morelos. Orquídea (Méx.) Volumen 16. México, D. F. 332 p.

Evans, M. 1990. Micropropagation: Axillary bud multiplication. Pp. 93-103 *in*: J.Y. Pollard & J. Walker (eds.). Methods in molecular biology. Vol. 6. Plant cell and tissue culture. Human Press. Clifton, New Jersey.

Faria, R., F. Rodríques, L. Oliveira & C. Müller. 2004. *In vitro Dendrobium nobile* plant growth and rooting in different sucrose concentrations. Horticult. Brasil., Brasilia. 22(4):780-783.

Fay, M. F. 1994. In what situations is *in vitro* culture appropriated to plant conservation? Biodivers. Conserv. 3: 176-183.

Fay, M., E. Bunn & M. Ramsay. 1999. *In vitro* propagation. A color Atlas of Plant Propagation and Conservation. Bowes, B. (ed.). The New York Botanical Garden Press. Bronx, New York. 224 p.

Fonnesbech, M. 1972. Organic Nutrients in the Media for Propagation of *Cymbidium in vitro*. Physiol. Plant. 27(3), 360-364.

Fu, F.M.L. 1978. Clonal propagation of *Aranda, Ascocenda*, and *Cattleya* by leaf tissue culture. Gardens Bull. (Singapore) 31: 132–138.

García-Cruz, J. & L. Sánchez. 1999. Orchidaceae II. *Epidendrum*. Flora de Veracruz. Fascículo 112. Instituto de Ecología, A. C. Xalapa, Ver. México

George, E. & Sherrington. 1984. Plant propagation by tissue culture. Handbook and directory of commercial laboratories. Exagetic Limited, England. 709 p.

George, P. & G. Ravishankor. 1997. *In vitro* multiplication of *Vanilla planifolia* using axillary bud explants. Pl. Cell Reports. 16(7):490-494.

Hágsater, E. 1971. Las Cattleyas. Orquídea (Méx.). 1(2): 3-8.

Hágsater, E. & A.M.A. Soto. 2001. Orchid conservation in México. Pp. 18-22 in: W.E. Higgins & B.W. Walsh (eds.) Orchid conservation proceedings. Selby Botanical Gardens Press, Sarasota.

Halbinger, F. & M. Soto. 1997. *Laelias* of Mexico. Orquídea (Méx.). 15(1): 37, 38, 40, 41, 50 y 61.

Hartmann, H., D. Kester & F. Davies Jr. 1990. Plant propagation. Fifth edition. Prentice Hall. New Jersey. 647 p.

IUCN/SSC Orchid Specialist Group. 1996. Orchids - Status Survey and Conservation Action Plan. IUCN, Gland Switzerland and Cambridge, UK.

Jiménez, M. R., S.L. Sánchez & J. García-Cruz. 1998. Familia Orchidaceae. Tribu Maxillarieae. Flora del Bajío y de regiones adyacentes. Fascículo 67. 83 p.

Ket, N., E. Hahn, S. Park, D. Chakrabarty & K. Paek. 2004. Micropropagation of an Endangered Orchid *Anoectochilus formosanus*. Biol. Plant. 48(3):339-344.

Krapiek, P., M. Milaneze & M. da Silva. 2003. Effects of different combinations of growth regulators for bud induction from seedlings of *Cattleya walkeriana* Gardner (Orchidaceae). Acta Scientiarum: Biological Sciences. Maringá, 25 (1):179-182.

Lackie, J. & J. Dow. 1999. The dictionary of cell and molecular biology. Third edition. Academic Press. P. 110.

Lo, K., K. Giles & V. Swhney. 1997. Acquisition of competence for shoot regeneration in leaf discs of *Saintpaulia ionantha x confusa* hybrids (African violet) cultured *in vitro*. Pl. Cell Rep. 15:416-420

Lozoya, S. H. 1985. Micropropagación de especies herbáceas. Pp. 65-79 *in*: M.L. Robe & V.M. Loyola (eds.). El cultivo de tejidos vegetales en México. CICY. México.

Lu, M. 2004. High Frequency Plant Regeneration from Callus Culture of *Pleione formosana* Hayata. Pl. Cell Tiss. Organ Cult. 78 (1):93-96.

Malabadi, R., G. Mulgund & N. Kallappa. 2005. Micropropagation of Dendrobium nobile from shoot tip sections. J. Pl. Physiol. 162(4): 473-478.

Martínez, A. 1985. Inducción *in vitro* de brotación múltiple en *Bletia urbana* Dressler (Orchidaceae) a partir de protocormos seccionados. Tesis profesional. Facultad de Ciencias. UNAM. México, D. F. 66 p.

Mata, R.M., A.V.M. Chávez, & B.R. Bye. 2001a. *In vitro* regeneration of plantlets from immature zygotic embryos of *Picea chihuahuana* Martínez, an endemic Mexican endangered species. In Vitro Cell Development Biology-Plant 37:73-78.

Mata, R. M., M.A. Monroy de la Rosa, G.K. Moebius & A.V. M. Chávez. 2001b. Micropropagation or *Turbinicarpus laui* Glass *et* foster, an endemic and endangered species. In Vitro Cell Developmental Biology - Plant 37:400-404.

Mata, M. & V. Salazar. 2003. Micropropagación y conserva-

ción de orquídeas mexicanas en el Jardín Botánico Clavijero. Lankesteriana 7:151-153.

Morel G. 1964. Régénération des Variétiés virosées par la culture des méristémes apicaux. Rev. Hortic. 136: 733–740.

Morel G. 1970. Neues auf den Gebiet der Meristem-Forschung. Die Orchidee 20: 433–443.

Mroginski, L. & W. Roca. 1993. Capítulo 2. Establecimiento de cultivo de Tejidos Vegetales *in vitro*. Cultivo de tejidos en la agricultura. Primera reimpresión. Centro Internacional de Agricultura Tropical (CIAT). Cali, Colombia. 969 p.

Murashige, T. & F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum 15:473-494.

SEMARNAT. 2002. Norma Oficial Mexicana NOM-059-ECOL-2001. Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Diario Oficial (6 de marzo 2002). México, D.F.

Oshiro M.A. & W.L. Steinhart. 1991. Preparation of protoplasts from cells of orchids representing various genera. *Lindleyana* 6: 36–41.

Park, S., E. Yeung, D. Chakrabarty & K. Paek. 2002. An efficient direct induction of protocorm-like bodies from leaf subepidermal cells of *Doritaenopsis* hybrid using thin-section culture. Plant Cell Reports. 21(1):46-51

Ospina, H. M. 1996. Orchidology and biotechnology. Orchids. October. 1072-1074.

Quintanar, A. 1961. Las plantas ornamentales, SAG. pp. 113-117.

Ramírez, C. 1990. Establecimiento del cultivo *in vitro* de orquídeas mexicanas en peligro de extinción. Tesis de Licenciatura (Biólogo). Facultad de ciencias. UNAM. México, D. F. 64 Págs.

Reinert, R.A & Mohr H.C. 1967. Propagation of *Cattleya* by tissue culture of lateral bud meristems. Proc. Amer. Soc. Horticult. Sc. 91: 664–671.

Rublúo, A. 1985. Estrategias para la preservación del germoplasma vegetal *in vitro*. In: M. Robert & Loyola (eds.). El cultivo de tejidos vegetales en México. Primera edición. CICY, CONACYT. 161 p.

Salazar R.V.M. 2003. Micropropagación de *Mormodes tuxtlensis* Salazar, *Cuitlauzina pendula* La Llave & Lex. y *Lycaste skinneri* (Batem. Ex. Lind.) lind. (Orchidaceae) a partir de protocormos. Tesis de licenciatura. Escuela de Biología. Benemérita Universidad Autónoma de Puebla. México. 106 p.

Sánchez-Espinoza, C., A. Jiménez, K. Moebius, A. Alvarez, V. Cervantes, I. Vargas, S. Luna, P. Ortega, A. Martínez, A. López, P. Olguín, M. Mata, M. Monroy, V. Chávez & R. Bye. 2000. Regeneración *in vitro* de especies mexicanas en peligro de extinción. Amaranto. 13(1): 12-19.

Segura, J. 2001. Fundamentos de fisiología vegetal. Capítulo 18. Introducción al desarrollo. Concepto de hormona vegetal. Azcon-Bieto, J y Talón, M. (Eds). 2da reimpresión. Mc Graw-Hill. Interamericana de España. Edicions Universitat de Barcelona. España. 522 p.

Serna, G.M.D. 1999. Biotecnología vegetal y conservación. Cuad. Biodivers. 2(1): 9-11.

Shyamal, K. & S. Pinaki. 2004. Regeneration of an Indigenous Orchid, Vanda teres (Roxb.) Lindl. Through In vitro Culture. Plant Tissue Culture.14 (1): 55-62 (2004).

Sosa V. & T. Platas. 1998. Extinction and persistence of rare orchids in Veracruz, México. Conservation Biology. 12(2): 451-455.

Soto, M. 1996. Orchids. *In*: A.M. Pridgeon (ed.). Unión Internacional para la Conservación de la Naturaleza y sus Recursos. Gland Switzerland. 153 p.

Soto, M. 2002. Plate 678 *in*: E. Hágsater & M.A. Soto (eds.). Icones Orchidacearum 5-6. Herbario AMO, México, D. F.

Stewart J. & J. Button. 1976. Rapid vegetative multiplication of *Epidendrum O'brienianum* in vitro and in the greenhouse. Amer. Orchid Soc. Bull. 45: 922–930.

Taiz, L. & E. Zeiger. 2002. Plant physiology. Third edition. Sinauer Associates, Inc. Publishers. Sunderland, Massachusetts. 690 p.

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BIOSYSTEMATIC STUDIES IN THE BRAZILIAN ENDEMIC GENUS *HOFFMANNSEGGELLA* H. G. JONES (ORCHIDACEAE:LAELIINAE): A MULTIPLE APPROACH APPLIED TO CONSERVATION

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KEY WORDS: Biosystematics, *Hoffmannseggella*, Laeliinae, endemic species, Campos Rupestres Vegetation, Conservation

Introduction

In recent molecular studies, the orchid genus *Laelia* Lindl. was segregated in two main groups: *Laelia* (a Mexican/Central American group), and *Sophronitis* Lindl. (a South American group) (van den Berg *et al*. 2000, van den Berg & Chase 2000). Later, other studies argued the legitimacy of this classification, suggesting the segregation of the South American group (*Sophronitis* sensu van den Berg *et al.* 2000) in four genera: *Hadrolaelia* (Schltr.) Chiron & V.P. Castro, *Hoffmannseggella* H.G. Jones, *Dungsia* Chiron & V.P.Castro, and *Microlaelia* (Schltr) Chiron & V.P.Castro (Chiron & Castro 2002).

Hoffmannseggella is one of the most ornamental genus of subtribus Laeliinae and has been shown to be monophyletic (van den Berg *et al.* 2000). It comprises exclusively rupicolous species and has a scattered distribution confined to the High Altitude Rocky Complexes (Brazilian Campos Rupestres and Campos de Altitude) (Semir 1991, but see discussion in Benites 2003) of Minas Gerais, Rio de Janeiro, Espírito Santo, and Bahia states. Chiron & Castro (2002) recognized 32 species in the genus, but the number has now increased to 42 (Castro & Chiron 2003, Chiron & Castro 2005, Lacerda & Castro 2005, Miranda & Lacerda 2003, Mota *et al*. 2003, Campacci 2005, Miranda 2005, Verola & Semir in press). Species delimitation is problematic, due to a great polymorphism in floral coloration and morphology, and the occurrence of natural hybrids (Blumenschein 1960a, 1960b, Brieger 1960, Barros 1990). This study aims at investigating the ecology and evolution of *Hoffmannseggella*, in order to provide the grounds for a more natural classification of the genus, and increase the knowledge necessary for the management and conservation of its species.

Material and methods

A multidisciplinary survey with emphasis on floral biology, breeding systems, pollination ecology, phylogeny, biogeography, and divergence times of 13 species of *Hoffmannseggella* is being conducted based on a varying number of populations.

Results and discussion

The majority of *Hoffmannseggella* species in this study does not normally produce nectar or floral odors, but this can vary among populations with fewer than 0.05% of the individuals in some populations with nectar and/or floral odor production). Generally, each flower lasts seven days on average and the anthesis is sequential racemose with about 3-7 available flowers at the same time. The species recorded so far are pollinated by small and inconstant Hymenoptera (belonging predominantly to families Apidae or Halichitidae), through deceit mechanisms. Bee pollination in *Hoffmannseggella* contradicts previous suggestions

(Brieger 1960a, Dressler 1981) which attribute a ornithophilous (in this case hummingbird) syndrome to these plants, but is in accordance with general trends in Laeliinae, where about 60% of species are pollinated by Hymenoptera (van der Pijl & Dodson 1966). The pollination by small bees in *Hoffmannseggella* can be interpreted as an evolutionary innovation in the subtribe, whereas pollination by large bees represents a plesiomorphic condition (Borba & Braga 2003, Smidt *et al*. 2006). This shift in pollinator type, accompanied by reduction in floral size and change in coloration patterns, was suggested to have occurred in response to the colonization of a new habitat, the Campos Rupestres (Blumeschein 1960a, Brieger 1960, 1961, 1966). Pollinators generally visit a single flower per inflorescence and the pollination mechanism observed fits the "gullet type" described by Dressler (1981). Visits are sporadic and diurnal, and although they may occur at any time they seem more frequent during warmer periods. Despite population isolation and pollinator scarcity, many species developed (or retained) spontaneous self-pollination mechanisms (Luer 1971, Cattling 1987, Knuth & Loew 1906) as a way to guarantee sexual reproduction in adverse situations (Catling 1990). The proportion of individuals with these mechanisms vary, with the highest number being found in small and isolated populations.

None of the species studied produced fruits through agamospermy, and fructification rate was generally low. A low rate of fruit formation has been observed in other deceptive orchids (Montalvo & Ackerman 1987, Ackerman 1989, Zimerman & Aide 1989) and can be an adaptation to limited resources (Schemske 1980, Montalvo & Ackerman 1987, Zimerman & Aide 1989). In these latter studies, species' survivor could only be assured by fruit formation with a high number of seeds (Dressler 1981, 1993).

Breeding systems were observed to vary from self-incompatibility to self-compatibility, depending on the species and population. Fructification and seed viability can vary considerably among populations of the same species. Grant (1975) and Lloyd (1979) pointed out that a great number of species can show mixed breeding systems, from exclusive selfing to outcrossing, including variations of those systems (Barrett *et al.* 2000, Lande & Schemscke 1985, Schemscke & Lande 1985). High seed viability was observed in interspecific crossings, sometimes reaching even higher levels of viability than in cross-pollination experiments. This corroborates the hybridization hypothesis proposed by Blumenschein (1960a, 1960b) and Brieger (1960). Hybridization between synchronopatric species, polyploidy (Blumenschein 1960a, 1960b, Brieger 1960, Barros 1990), and disploidy (see Costa 2006 for a discussion on the same species and populations included in this work) seem to be the main mechanisms triggering radiation in the genus.

Estimates of divergence times in *Hoffmannseggella,* based on molecular sequence variation, indicate a recent diversification event. The crown age of the genus is placed in the Latest Miocene, and the short ages of several species imply that speciation is still occurring at a high rate (Verola *et al*. unpubl. data). A high speciation rate seems correlated with the climate oscillation that characterized the Pliocene and Pleistocene, causing the expansion and retraction of open vegetation types (Ledru 2002, Safford 1999). These events promoted high allopatric speciation by vicariance during wet periods, when populations became isolated in the few patches of open vegetation confined to mountain tops. As climate became drier, the expansion of open habitats and establishment of migratory corridors promoted contact between previously isolated species and thus facilitated sympatric speciation (mainly hybridization and polyploidy) (Verola *et al.* unpubl. data). A similar speciation mechanism has been proposed by other authors, such as Alves & Kolbek (1994), Pirani *et al.* (1994), and Semir (1991).

Threats and conservation status

Similarly to many other plant groups occurring in the Brazilian campos rupestres (Giullieti *et al.* 2005), the current status of conservation of the species in *Hoffmannseggella* is precarious. The majority of its species seem to be endangered, as they grow outside protected areas and are thus subjected to grazing, fires, illegal collecting, and habitat destruction. As many as 47% of the species are micro-endemic (with only one natural population known), and some are known only from the type collection.

The highest species diversity is found in the state of Minas Gerais, in the proximities of Belo Horizonte city. The area is densely populated and comprises a large number of ore prospection fields. Considering the isolation of the area, its high level of endemicity, and poor availability of pollinators, coupled with the great anthro-

pological pressure on natural populations, the populations occurring in this area appear highly subjected to stochastic events, which may lead to extinction. We therefore suggest the delimitation of fifteen new areas for the conservation of the group's total diversity. These areas were designed considering present and future climate conditions (obtained by the Ecological Modelling Techniques of Beaumont *et al.* 2005, Hijmans *et al.* 2004, and Hijmans *et al.* 2005) as well as centers of endemism (inferred by Parsimony Analysis of Endemicity; Morrone & Crisci 1995). The suggested areas lay outside current conservation areas, which emphasizes the necessity of an immediate establishment. This study urges for additional works on other taxa characteristic of the Brazilian Campos Rupestres, to better identify regions of range overlaps and thus increase the total number of species per conservation area.

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LITERATURE CITED

Ackerman, J.D. 1989. Limitations to sexual reproduction in *Encyclia krugii* (Orchidaceae). Syst. Bot. 14: 101-109.

Alves, R.J.V & J. Kolbek.1994. Plant species endemism in savanna vegetation on table mountains (Campo Rupestre) in Brazil. Vegetatio 113: 125-139.

Barrett, S.C.H., A.M. Baker & L.K. Jesson. 2000. Matting strategies in Monocotyledons. Pp. 258-269 *in*: K.L. Wilson & D.A. Morrison (eds.). Monocots: Systematics and Evolution. CSIRO, Melbourne, Australia.

Barros, F. 1990. Diversidade taxonômica e distribuição geográfica das Orchidaceae brasileiras. Acta bot. bras. 4: 177-187.

Beaumont, L.J., L.Hughes, & M.Poulsen. 2005. Predicting species distribution: use of climatic parameters in BIOCLIM and its impact on predictions of species' current and future distributions. Ecol. Model. 186: 250-269.

Benites, V.M., A.N. Caiafa, E.S. Mendonça, C.E. Schaefer & J.C.Ker. 2003. Solos e vegetação nos complexos rupestres de altitude da mantiqueira e do espinhaço. Floresta e Ambiente. 10 (1): 76-85

Blumenschein, A. 1960a. Estudo sobre a evolução no subgênero *Cyrtolaelia* (Orchidaceae). Livre Docente diss., Universidade de São Paulo / Escola Superior de Agricultura "Luiz de Queiroz"/Esalq, Piracicaba, São Paulo, Brazil.

Blumenschein, A. 1960b. Número de cromossomas de algumas espécies de orquídeas. Publicação Científica do Instituto de Genética, Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz" 1: 34-44.

Borba, E.L. & P.I.S. Braga. 2003. Reproductive biology of *Pseudolaelia corcovadensis* (Orchidaceae): melittophyly and self-compatibility in a basal Laeliinae. Rev. Bras. Bot. 26: 541-549.

Brieger, F.G. 1960. Contribuições para a taxonomia das orquídeas. Publicações Científicas do Instituto de Genética, Esalq, USP 1: 1-31.

Brieger, F.G. 1961. A evolução filogenética nos trópicos. Pp. 154-161 *in*: F.G. Brieger *et al.* (eds.). Atas do Primeiro Simpósio Sul Americano de Genética. Cadeira de Citologia e Genética Geral da Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, São Paulo, Brazil.

Brieger, F.G. 1966. Evolução filogenética, com referência especial às plantas superiores. Pp. 464-515. in Pavan, C. & Da Cunha, A.B. eds. Elementos de Genética. Companhia Editora Nacional & Universidade de São Paulo, São Paulo, Brazil.

Campacci, M.A. 2005. Duas novas espécies de orquídeas do Brasil. Boletim CAOB 57: 24-31.

Castro Neto,V.P & G.R. Chiron. 2003. Une nouvelle especè d´*Hoffmannseggella* (Orchidaceae) du Brésil. Richardiana 3 (1): 64-68.

Catling, P.M. 1987. Notes on the breeding systems of *Sacoila lanceolata* (Aublet) Garay (Orchidaceae). Ann. Mo. Bot. Gard.74: 58-68.

Catling, P.M. 1990. Auto pollination in Orchidaceae. Pp. 121-158 *in*: J. Arditti (ed.). Orchid Biology, reviews and perspectives, V. Timber Press, Portland, U.S.A.

Chiron, G.R. & V.P. Castro Neto. 2002. Révision des espèces brésiliènnes du genre *Laelia* Lindley. Richardiana 2:4-28.

Chiron, G.R. & V.P. Castro Neto. 2005. Contribution à la connaissance des orchiées du Brésil. VI – Une nouvelle espèce de *Hoffmannseggella* du Minas Gerais (Brésil). Richardiana 5 (1): 7-14.

Costa, J.Y. 2006. Citotaxonomia e aspectos evolutivos de espécies de *Hoffmannseggella* H.G.Jones (Orchidaceae) de campos rupestres brasileiros. Ph. D. diss., Instituto de Biologia, Universidade Estadual de São Paulo, Campinas, Brazil.

Dressler, R.L. 1981. The orchids: natural history and classification. Harvard University Press, Cambridge.

Dressler, R.L. 1993. Phylogeny and classification of the orchid family. Cambridge University Press, Cambridge.

Giullieti, A.M., R.M. Harley, , L.P. Queiroz, , M.G. Wanderley & C. Van den Berg. 2005. Biodiversidade e conservação das plantas no Brasil. Megadiversidade 1 (1): 52-61.

Grant,V. 1975. Genetics of Flowering Plants. Columbia University Press, New York.

Hijmans, R.J., S.E. Cameron, J.L. Parra, P. G. Jones & A. Javis. 2004. The WorldClim interpoled global terrestrial climate surfaces. Version 1.3. http://biogeo.berkeley.edu/worldclim/worldclim.htm.

Hijmans, R.J., L. Guarino, A. Jarvis, R. O´Brien, P. Mathur, C. Bussink, M. Cruz, I. Barrantes & E. Rojas. 2005. Diva-

GIS. Version 5.2. http://www.diva-gis.org/docs/DIVA-GIS5.manual.pdf.

Knuth, P. & E. Loew. 1906. Handbook of flower pollination. Claredon Press, Oxford.

Lacerda Jr., K.G. & V.P. Castro Neto. 2005. Deux nouveaux taxons de *Hoffmannseggella* du Minas Gerais (Brésil). Richardiana 5 (1): 15-25.

Lande, R. & D.W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39:24-40.

Ledru, M.P. 2002. Late Quaternary history and evolution of the cerrados as revealed by palynological records. Pp. 33-50 *in*: P.S. Oliviera & R.J. Marquis (eds.). The cerrados of Brazil. Columbia University Press, New York, USA.

Lloyd, D.G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. Amer. Natur. 113:67-79.

Luer, C.A. 1971. Abnormal development of the anther – a report of two cases. Florida Orchidist 13: 26-29.

Miranda, F. & Jr., K.G. Lacerda. 2003. Studies in Brazilian Laeliinae. Part 1: New species and natural hybrids in *Hoffmannseggella*. Orchids 72 (11): 848-857.

Miranda, F. 2005. Studies in Brazilian Laeliinae. Part 2: A new species in *Hoffmannseggella*. Orchids 74 (6): 458-461.

Montalvo, A.M. & J.D. Ackerman. 1987. Limitations to fruit production in *Ionopsis utricularioides* (Orchidaceae). Biotropica 19: 24-31.

Morrone, J.J. & J.V. Crisci. 1995. Histórical Biogeography: Introductin to Methods. Annu. Rev. Ecol. Syst. 26: 373-401.

Mota, R.C., P.L. Viana & K.G. Lacerda Jr. 2003. *Hoffmannseggella pendula*, une nouvelle espèce d'Orchidaceae du Brésil. Richardiana 4 (1): 1-8.

Pirani, J.R., A.M. Giulietti, R. Mello-Silva & M. Meguro. 1994. Checklist and patterns of geographical distribution of the vegetation of Serra do Ambrósio, Minas Gerais, Brazil. Rev. Bras. Bot. 17 (2): 133-147.

Safford, D.S. 1999. Brazilian Páramos I. An introduction to the physical environment and vegetation of the campos de altitude. J. Biogeogr. 26: 693–712.

Schemske, D.W. & R. Lande. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. Evolution 39:41-52.

Schemske, D.W. 1980. Evolution of floral display in the orchid *Brassavola nodosa*. Evolution 34: 489-493.

Semir, J. 1991. Revisão Taxonômica de *Lychnophora* Mart. (Vernonieae:Compositae). Ph. D. diss., Instituto de Biologia, Universidade Estadual de São Paulo, Campinas, Brazil.

Smidt, E.C., V. Silva-Pereira & E.L. Borba. 2006. Reproductive biology of two *Cattleya* (Orchidaceae) species endemic to north-eastern Brazil. Plant Species Biology 21: 85-91.

van den Berg, C. & M.W. Chase. 2000. Nomenclatural notes on Laeliinae - I. Lindleyana 15: 115-119.

van den Berg, C., W.E. Higgins, R.L. Dressler, W.M. Whitten, M.A.S. Arenas, A. Culham & M.W. Chase. 2000. A phylogenetic analysis of Laeliinae (Orchidaceae) based on sequence data from internal transcribed spaces (ITS) of nuclear ribosomal DNA. Lindleyana 15: 96-114.

van der Pijl, L. & C.H. Dodson. 1966. Orchid flower: their pollination and evolution. University of Miami Press, Coral Gables.

Verola, C.F. & J. Semir. 2007. *Hoffmannseggella viridiflora* (Orchidaceae, Laeliinae), A New Species from Brazilian Campos Rupestres. Novon (accepted)

Zimmerman, J.K. & T.M. Aide. 1989. Patterns of fruit production in a neotropical orchid: pollinator vs. resource limitation. Amer. J. Bot. 76: 67-73.

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MICRO-ENVIRONMENT CONDITIONS, MYCORRHIZAL SYMBIOSIS, AND SEED GERMINATION IN *CYPRIPEDIUM CANDIDUM*: STRATEGIES FOR CONSERVATION

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KEY WORDS: *Cypripedium candidum*, mycorrhizal, environment, terrestrial, rhizomes

Introduction

Cypripedium candidum Muhl. ex Willd. (White Lady's Slipper), a terrestrial northern United States / southern Canada prairie orchid, reproduces both by seed and adventitious buds from older roots and rhizomes. To determine the pollination effects of diverse prairie micro-environments caused by competing foliage, particularly with respect to height and density of surrounding flora, ovary development (pollination success) versus ovary abortion (no pollination) was recorded for every flower in three diverse populations in eastern South Dakota, USA. Dense and medium-dense competing foliage populations correlated with similar orchid seed sets (22%, n=74 and 18%, n=615, respectively), while the more open, exposed orchid population demonstrated a significant increase in success (44%, n=258). In addition, a separate, hand-pollinated population in an open location (n=49) resulted in 100% ovary development. Samples of developing ovaries were histologically examined and more extensive ovule development was observed in ovaries from these open sites. Since micro-environments of tall, dense, competing foliage seem to limit sexual reproduction in *C. candidum* by physically preventing pollinators from accessing the flowers, this orchid also relies on clonal reproduction via its rhizomes. It may also rely on mycorrhizal fungi to receive necessary nutrients and / or enzymes for plant growth to compensate for reduced photosynthates. To locate putative orchid-fungal associations, histological studies of roots and rhizomes were completed. No fungal pelotons were observed in root or rhizome samples harvested in early summer (end of May), but were clearly visible in root cortical cells six to eight weeks later. These differing levels of mycorrhizal development may be a function of continued or new fungal and / or root growth, or seasonal changes in the soil environment. Further studies of mycorrhizal development throughout the growing season, as well as fungal identification and symbiotic seed germination, are currently underway to help identify these intricate inter-specific associations necessary for the survival and conservation of *C. candidum* in eastern South Dakota.

Description

Cypripedium candidum (White Lady's Slipper) is a perennial terrestrial orchid that is indigenous to 13 states in north-central and north-east United States and southern regions of Ontario and Manitoba, Canada. *Cypripedium candidum* produces solitary stems (10-30 cm), as well as dense clonal clumps, from its short rhizomes and fibrous roots. Each stem produces several strongly ribbed, densely pubescent leaves (5-16 cm x 1.5-6 cm) that are typically held nearly erect, sheathing the stem and overlapping at the base. The uppermost leaf (elliptical, green foliaceous bract) is smaller than the other leaves, standing erect, subtending the flower and sheathing the ovary. The stems are usually terminated by a single flower (occasionally two) characterized by its ivory-white egg-shaped pouch (labellum) for which it was named (from the Latin *Cypris* for "Venus", *pedilon* for "shoe" and *candidum* for "shining white") (Fig. 1). This white shiny or waxy appearing lip petal (1.7-3.3 x 1-1.5 cm) has a rounded opening above with in-rolled edges and may be delicately lined with purple veins toward the bottom and slightly purple-spotted

FIGURE 1. *Cypripedium candidum* (White Lady's Slipper) flower (Wake 2004).

FIGURE 2. *Cypripedium candidum* developing ovary following successful pollination (Wake 2004).

around the pouch opening. The two lateral petals (4-5 cm), which are similar to the sepals, are pale yellow-greenish with lavender veins, pointed, and spirally twisted. The dorsal sepal (2-2.5 cm) is ovate to elliptical and the two lateral sepals are united nearly to the apex. The staminodes, ovate in shape, appear yellow with purple spots and are attached to the style and stigma forming the column. The stigma is borne on the lower side near the base of the column and the pollen is shed in bilateral waxy pollinia. The ovary is inferior, 3-celled, (Fig. 2) and matures into an ellipsoid dehiscent capsule. The seeds are very numerous and minute in size. *Cypripedium candidum* are thought to be pollinated by adrenid and halictid bees (Catling and Knerer 1980).

Habitat

Cypripedium candidum is not a shade tolerant plant and prefers open, wet, rich prairie meadows with alkaline soils and calcareous fens (Galbraith 1996). However, prairie habitats have been transformed by agricultural and urban development, through cropland expansion, extensive grazing, and wetland drainage. Even in remaining natural areas, the control of wildfires may be endangering the white lady's slipper habitat. In natural ecosystems, wildfires curtail plant succession and prevent development of over-shady conditions that are detrimental to continued *C. candidum* growth and survival (USGS).

Objectives

This project was designed to study the effects of different micro-environments caused by competing foliage, particularly with respect to pollination and ovary / seed growth and development of *C. candidum* on three habitats in eastern South Dakota, USA.

Methods

During the summer of 2003, three different *C. candidum* populations were examined during seed-set and ovary-development stages of the life cycle for sexual reproduction success in relation to diverse surrounding vegetation micro-environments, particularly with respect to height and density of the surrounding flora. By midsummer, flowers were senescing and ovary development in pollinated individuals was evident. The Dense Competing Foliage (DCF) site is a

FIGURE 3. *Cypripedium candidum* senescing / aborting ovary tissue following no pollination (Wake 2004).

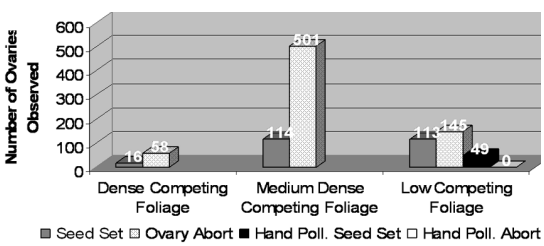

FIGURE 4. Comparisons of *C. candidum* seed set versus ovary abortion in dense competing foliage (DCF), medium-dense competing foliage (MDCF) and low competing foliage (LCF) sites.

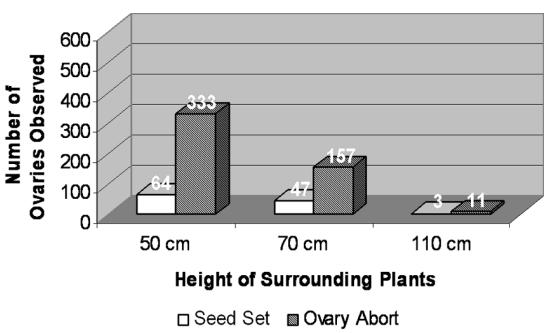

FIGURE 5. Effects of competing foliage heights for seed set in *C. candidum* in medium-dense competing foliage (MDCF) site.

railroad right-of-way very densely populated by brome grass (*Bromus inermis*), a perennial cool-season sod grass with vigorous rhizomes, and the site had not been mowed for many years. The Medium-Dense Competing Foliage (MDCF) site is a federal wildlife production area populated by mixed prairie grasses, forbs and thickets. It had not been grazed for three years, resulting in a range of dense to medium-dense shade / competition for the intermingled orchid population. The Low Competing Foliage (LCF) site is adjacent to a federal waterfowl production area, and, by contrast, was mowed late the previous fall (2002) so the orchids were growing in a much more open and exposed environment.

To compare the pollination success between these different growing conditions, ovary development (successful pollination) (Fig. 2) vs. ovary abortion (no pollination) (Fig. 3) was recorded for every flower located.

Results

The more shaded DCF and MDCF sites demonstrated only 22% (n=74) and 18% (n=615) seed set, respectively, while at the more open LCF location, successful seed set was 44% (n=258) (Fig. 4). In addition, a separate, hand pollinated population at the open LCF site (n=49) resulted in 100% ovary / seed development. To further assess the affects of height, as well as density, of surrounding plants in the dense to medium-dense competing foliage (MDFS) site, this area was subdivided by height of surrounding foliage, and ovary development quantified (Fig. 5). In areas averaging 50 cm tall competing foliage, the seed set was 16% (n=397); 70 cm tall, it was 23% (n=204); and in 110 cm grass, it was 21% (n=14). Within this medium-density micro-environment, with a limited number of orchid flowers, differences between surrounding plant height could not be differentiated.

Discussion

Environmental factors, such as temperature, soil type, and light exposure, affect the distribution of *C. candidum*, due to the fact that like many orchids, it is highly specialized in its requirements (Galbraith 1996). Data presented in Fig. 4 demonstrates that a micro-environment of dense, competing foliage can

also affect sexual reproduction in *C. candidum* by physically preventing pollinators from accessing the flowers. Tall, dense surrounding plants would also limit wind-borne seed dispersal, and possibly seed germination. Other habitat degradation threats to consider are trampling of plants and delicate root / rhizomes in the moist soils by over-grazing and all-terrain-vehicle use. Mowing or controlled burns help open the micro-environment to more light for the orchids, but they also remove the thick layer of leaf litter that protects over-wintering rhizomes and next year's fully-formed stem shoots through the harsh northern prairie winters. Even though orchids are able to reproduce vegetatively by underground rhizomes, sexual reproduction provides the critical genetic diversity to insure survival in changing environments. Further study of flower pollination, soil / cultural needs and mycorrhiza-seed germination associations of this native orchid, in relation to land-use management practices, will be useful in protecting this rare natural resource.

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LITERATURE CITED

Bowles, M.L. 1983. The tallgrass prairie orchids *Platanthera leucophaea* (Nutt.) Lindl. and *Cypripedium candidum* Muhl. ex Willd: some aspects of their status, biology and ecology, and implications toward management. Natural Areas Journal 3(4): 14-37.

Catling, P.M. & Knerer, G. 1980. Pollination of the small white lady's-slipper (*Cypripedium candidum*) in Lambton County, southern Ontario. Can. Field-Nat. 94: 435-438.

Galbraith, D.A. 1996. Endangered Plants of Ontario. www.science.mcmaster.ca

USGS, 2003. www.npwrc.usgs.gov/resource/literatr/wild-flwr/species/cyprcand.htm

Wake, C.M.F. 2004. All *Cypripedium candidum* photos used in this abstract.

Carol M.F. Wake received her PhD in plant physiology and anatomy and currently teaches botany, plant anatomy, and general biology at South Dakota State University, Brookings, SD, USA. Carol is particularly interested in research encompassing the micro-environmental requirements of *Cypripedium candidum*, an indigenous terrestrial orchid of South Dakota. Several recent projects have been conducted in collaboration with undergraduate botany / plant anatomy students.

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HARVESTING MYCORRHIZAL FUNGI: DOES IT PUT *CALADENIA* PLANTS IN PERIL?

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KEY WORDS: mycorrhizal harvesting, isolation, *Caladenia,* emergence, reproduction

The 'slice' method for harvesting mycorrhizal fungi from terrestrial orchids has been suggested for use with endangered species (Dixon 2004, Stewart 2004). It involves uncovering the mycotrophic region (containing mycorrhizal infection) of a plant and removing a slice of tissue for fungal isolation. This method is less destructive than removing whole mycotrophic parts or whole plants, which are the most common published methods of harvesting orchid mycorrhizal fungi. The 'slice' method is especially useful for *Caladenia* species, as their mycotrophic tissue occurs in a swollen collar beneath the leaf, which, when removed totally stops photosynthesis and reproduction in a given season. At this stage, there are no published studies using this potentially useful method for harvesting *Caladenia* mycorrhizal fungi. Lack of evidence to support the use of this technique has limited its application in the symbiotic propagation and conservation of endangered terrestrial orchids. The focus of this study therefore was to examine the effect of the 'slice' method on orchid emergence and reproduction using a relatively common species, *Caladenia tentaculata* Schltdl.

Preliminary experiment

A preliminary experiment was undertaken at a C. *tentaculata* population located near Maldon, Victoria. In 2004, 99 adult plants were marked for monitoring and mycorrhizal fungi was harvested from six of these plants at budding stage (winter) and from ten plants at flowering stage (spring). Tissue slices were collected from each plant by removing soil to 1cm below the stem-collar and taking a 2-3 mm slice from the rinsed stem-collar with a sterile scalpel blade. The life stage of each marked plant was recorded in winter (emergent or non-emergent) and again in spring (flowering or non-flowering) for three growing seasons, commencing in 2004. A fungal isolation experiment identified a culture method with a 100% success rate. This method involved surface sterilising the tissue slices with 0.5% NaOCl for 30 seconds and plating fungal pelotons (as in Rasmussen *et al.* 1990) onto the Fungal Isolating Medium (FIM, Clements *et al*. 1986) with 0.05% streptomycin. All isolations were undertaken within 24 hours of harvesting. One fungal isolate from each plant successfully cultured was tested in its ability to germinate *C. tentcualata* seed. All isolates tested germinated seed, thus confirming their mycorrhizal status.

Initial results suggested that the season of harvest affected the subsequent emergence of harvested plants. The 2005 emergence results revealed that significantly more plants from which mycorrhizal fungi were harvested in spring (2004) emerged (100%) than those harvested in winter (50%) of the same year. However, the 2006 emergence results did not support the initial finding as the emergence of the plants harvested at the two time intervals was the same (66%). In neither year did the emergence of the harvested plants differ significantly from that of the un-harvested plants. This lack of significant differences indicates that harvesting mycorrhiza from *Caladenia* plants using the 'slice' method does not affect subsequent plant emergence within two years.

Multiple population experimentation

Adult plants from six geographically distinct *C. tentaculata* populations in Victoria (located near

Angelsea, Chewton, Inverleigh, and Maldon) were marked for monitoring. Thirty-three plants from the Anglesea population, 30 from the Inverleigh population, 29 from the Chewton population and 83 plants from the Maldon population were marked. In 2005, six adult plants from six populations (including un-monitored populations at Wonthaggi and Eltham) were harvested using the 'slice' method in winter and from the four monitored populations in spring. Half of the harvested plants from each site were hand pollinated and the seed was collected for viability testing, using fluorescein diacetate (FDA) staining. Plants were monitored in winter and spring for two growing seasons. Fungal isolates were successfully obtained from all but one of the 36 plants harvested in winter using the method optimised in the preliminary experiment. At least one isolate from each successfully cultured plant was tested to determine its ability to germinate seed. The germination experiments, part of a large fungal diversity study, confirmed the mycorrhizal status of all but one of 49 isolates.

A proportion of the emergent un-harvested plants at all four populations had died back before the spring monitoring in 2005. These plants lost some photosynthetic and complete reproductive function for the 2005 season. Analysis of the spring 2005 monitoring data revealed a significant difference between the proportion of harvested (44%) and un-harvested (86%) plants that remained above ground in the Maldon population. There was no such difference in the three other monitored populations. This indicated that the effect of the 'slice' method within the year of harvest differed between *C. tentaculata* populations. The 2006 emergence results showed no significant difference between plants harvested in winter (68%) and those harvested in spring (66%) supporting the 2006 results of the preliminary experiment. As these results were obtained using a larger sample size across a number of environments, they are more reliable than the initial findings. They clearly show that there is no difference in the effect on emergence between the seasons of harvest.

Emergence of harvested and un-harvested plants did not differ significantly at any of the four monitored populations. This supports the findings of the preliminary experiment and clearly shows that harvesting does not affect the subsequent emergence of *Caladenia* plants. There was no effect of harvesting on the flowering observed in *C. tentaculata* populations in this study. The proportions of harvested and un-harvested plants that flowered in the four monitored populations in spring 2005 were not significantly different. Harvesting did not significantly affect seed viability, which was 54% for harvested plants and 58% for un-harvested plants across all six populations. Analysis of the spring monitoring and seed viability data suggests that harvesting did not negatively effect reproduction in the year of harvest.

Endangered taxa

In 2005 mycorrhizal fungi was harvested from single plants of two endangered *Caladenia* taxa, *C.* sp. aff. *fragrantissima* (central Victoria) sensu Bishop (1996) and *C.* sp. aff. *fragrantissima* (Inverleigh) sensu Ross (2000). The same methodology was used as above. Both harvested plants emerged in 2006. The isolations from both endangered *Caladenia* taxa were successful and isolates from both taxa were used to germinate seed for *ex situ* cultivation and re-introduction. All the isolates tested were able to germinate seed from the taxa they were harvested from. These results show that the 'slice' method can be effectively used on endangered *Caladenia* taxa.

Conclusions

This study has shown that the 'slice' method of harvesting can be used to successfully isolate mycorrhizal fungi from *Caladenia* plants. It has also shown that this method can be used both in large fungal diversity studies with common species and to obtain mycorrhiza for the propagation of endangered taxa for direct conservation purposes. Harvesting mycorrhiza using this method does not affect the emergence of *Caladenia* plants up to two years after harvesting. Initial results suggested that spring harvesting had less affect on subsequent plant emergence than winter harvesting. Further experimentation across multiple *C. tentaculata* populations revealed that there was no difference in the effect between the two harvesting times. No negative effect of harvesting on reproduc-

tion of *Caladenia* plants was recorded. The effect of the 'slice' method on plant function was shown to differ between *C. tentaculata* populations within the year of harvest.

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LITERATURE CITED

Clements, M., P.J. Cribb & H. Muir. 1986. A preliminary report on the symbiotic germination of European terrestrial orchids. Kew Bull. 14: 437-445.

Dixon, K.W. 2004. Orchid re-introduction workshop, 2nd International Orchid Conservation Congress, Sarasota, Florida.

Rasmussen, H.N., T.F. Andersen & B. Johansen. 1990. Temperature sensitivity of *in vitro* germination and seedling development of *Dactylorhiza majalis* (Orchidaceae) with and without a mycorrhizal fungus. Pl. Cell Environ. 13: 171-177.

Stewart, S.L. 2004. Mycorrhizal workshop, 2nd International Orchid Conservation Congress, Sarasota, Florida.

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SITE AMELIORATION FOR DIRECT SEEDING OF *CALADENIA TENTACULATA* IMPROVES SEEDLING RECRUITMENT AND SURVIVAL IN NATURAL HABITAT

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KEY WORDS: *in situ* seed germination, site amelioration, *Caladenia*

The genus *Caladenia* contains the largest number of threatened orchid species in Australia and improving the success of re-introductions would allow existing populations to be strengthened and new populations to be established. Batty *et al.* (2006) showed that direct seeding of *C. arenicola* Hopper & A.P. Br. into habitat soil inoculated with mycorrhizal fungus resulted in a good *in situ* germination rates. However, these seedlings did not survive the summer dormancy. In Victoria, there have been successes with conservation of endangered *Caladenia* species using direct seeding and intensive site management (Jeanes and Backhouse 2000). Successes in these instances have been attributed to the following site amelioration treatments: soil disturbance, addition of organic matter to *in situ* soil and supplementary watering. It is possible that all of these treatments have contributed to successful seedling recruitment and survival. However, due to the species in these cases being endangered, little seed has been available for scientific experimentation and it has been difficult to tease out which treatments singly or in combination, provide optimum conditions for seed germination and seedling survival.

Caladenia amoena: a critically endangered orchid

Over a period of four years, a site in a public reserve was prepared for a translocation of *C. amoena* D.L. Jones from a remnant population on nearby private land. The proposed translocation site was steeply sloped and had suffered from topsoil erosion caused by high grazing pressure and herbivore traffic. The site was fenced and prepared by building up a flattened ledge to trap moisture from natural rainfall and the addition of natural organic matter over the four year period. In May 2004, 15 adult *C. amoena* plants were translocated to the site. The soil was loosened to a depth of 8 cm to allow planting of the soil plugs containing the plants. Seed was also sown into this loosened soil. The translocated plants and emergent seedlings were watered, by misting, when site conditions were dry. Seed has been sown around the translocated plants each year from 2004 to 2006. In the subsequent years following translocation soil was disturbed to a depth of 25 mm at each seed sowing.

In August 2004, 26 seedlings emerged and remained above ground for 12 weeks. Of these seedlings, 83% survived to emerge in 2005. Ten new seedlings emerged in August 2005 and remained above ground for 10 weeks. Only 40% of them survived to emerge in 2006. Seedlings with leaf lengths under 10 mm rarely survived in either year. The mean leaf length was 15.8 mm for the seedlings that survived and 8 mm for those that did not. Ten new seedlings emerged in 2006 and remained above ground for only eight weeks. The percentage survival of these seedlings will be calculated when they emerge in 2007. It is suspected that less than 40% will survive, as it appears that the shorter the growing season of the new seedlings, the fewer of them sur-

vive. The growing season of the new seedlings has dropped by two weeks every year from 2004 to 2006. This may be due to increasingly drier site conditions during each consecutive year of a prolonged drought experienced in south-eastern Australia.

Caladenia tentaculata: a relatively common species

In 2004, experimental plots in the natural habitat of *C. tentaculata* Schltdl. were tested in the absence of adult plants for the occurrence of mycorrhizal fungi by seed baiting (Rasmussen and Whigham 1993). As it has been shown that mycorrhizal fungi occurs around adult orchid plants (Perkins and McGee 1995, Batty *et al.* 2001, IIyes *et al.* 2005), seed baits were buried along transects through three nearby populations of *C. tentaculata* as positive controls. Positive seed baits were only exhumed from one of the three transects, which was through a population of *C. tentaculata* in a site with a thick layer of *Banskia marginata* vegetative litter. The other two transects were located in populations which lacked any substantial litter layer. Batty *et al*. (2001) found that distribution of positive germinates in seed baiting experiments in Western Australia were correlated with the presence of leaf litter. They suggested that the presence of leaf litter may increase soil moisture and provide the mycorrhizal fungi with a suitable substrate. The *in situ* seed baiting method (Rasmussen and Whigham 1993) does not only test for the presence of mycorrhizal fungi, but also whether the soil environment is appropriate for natural seed germination.

Only 8 of 800 baits exhumed from the experimental plots were positive and were they randomly distributed. These results indicated that either there was little naturally occurring mycorrhiza present or that the conditions in the experimental plots did not enhance seed germination. As Batty (2001) had shown that the *in situ* seed germination rate increased with amount of mycorrhizal inoculum, the decision was made to inoculate the plots with mycorrhizal fungi when direct seeding. A mycorrhizal fungus was isolated from an adult plant in the vicinity (as in Rasmussen *et al*. 1990), shown to germinate *C. tentaculata* seed *in vitro* (as in Clements *et al.* 1986), and then used to inoculate sterilised millet seed. Seed germination was also successfully tested *in vitro* using infected millet seed as an inoculum. In May 2005, the experimental plots were ameliorated by adding naturally occurring organic matter, disturbing the soil and watering to supplement monthly average rainfall. The treatments used in this study included each of the three amelioration types alone and in every combination with a negative control of no amelioration. The experimental treatments were randomised according to site conditions and the mean percentage imbibition of the seed in each experimental plot as calculated in the 2004 seed baiting experiment.

After 19 weeks 56 seedlings emerged and their leaf lengths and widths were measured fortnightly during their 10 week growing season. The mean maximum leaf length reached was 8.5 mm. Initial analysis revealed that the mean number of emergent seedlings in the plots subjected to all three amelioration types (3 seedlings) was significantly greater than that in the negative control plots (0 seedlings). This result clearly indicates that *in situ* seedling recruitment is enhanced by site amelioration. Further analysis of the data showed that there was a significant effect of soil disturbance on seedling emergence ($p=0.03$) and evidence of an effect of addition of organic matter ($p=0.09$). The analysis showed no significant effect of watering to supplement monthly rainfall. In Victorian bush land habitats soil compaction has been recognised as a problem threatening orchid populations (Backhouse and Jeanes 1995). The results of this study show that relieving soil compaction through disturbance enhances orchid seedling recruitment *in situ*.

In 2006, 16% of the seedlings emerged after the summer dormancy. The mean maximum leaf length reached in 2005 by the surviving seedlings was 11.1 mm, where it was 8.0 mm for the seedlings that did not survive. All but one of the second year seedlings emerged in plots with soil disturbance as one of the site amelioration types. Although not enough seedlings survived to conduct statistical analysis on the effect of the treatments on survival it is likely that soil disturbance was a critical factor. Soil disturbance may allow seedlings to germinate deeper in the soil profile and/or increased their dropper (tuber stalk) length. Both of these factors would result in first year tubers developing deeper in the soil than those growing in non-disturbed treatments. Tubers that are deeper in the soil profile would have more protection from desiccation during the summer dormancy.

Conclusions

This study has shown, for the first time that *in situ* seedling recruitment of *Caladenia* species can be improved by site amelioration. Soil disturbance was the most effective treatment at improving recruitment of *C. tentaculata in situ*. There was evidence that soil disturbance also improved seedling survival during the summer dormancy. The leaf length reached by new seedlings in their first year of growth and the length of their growing season also effected their subsequent survival. This information will directly benefit the conservation and reintroduction of Australia's most endangered genus.

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LITERATURE CITED

Backhouse, G. N. & J.A. Jeanes. 1995. The Orchids of Victoria. The Meigunyah Press, Melbourne.

Batty, A.L. 2001. The role of symbiotic seed germination and seed ecology in the conservation of selected Western Australian terrestrial orchids. PhD Thesis, Soil Science and Plant Nutrition, The University of Western Australia.

Batty, A.L., K.W. Dixon, M.C. Brundrett & K. Sivasithanparam. 2001. Constraints to symbiotic germination of terrestrial orchid seed in a mediterranean bushland. New Phytol. 152: 511-520.

Batty, A.L., M.C. Brundrett, K.W. Dixon & K. Sivasithanparam. 2006. *In situ* symbiotic seed germination and propagation of terrestrial orchid seedlings for establishment at field sites. Austral. J. Bot. 54: 375-381.

Clements, M., P.J. Cribb & H. Muir. 1986. A preliminary report on the symbiotic germination of European terrestrial orchids. Kew Bull. 41: 437-445.

IIyes, Z., S. Rudnoy & Z. Bratek. 2005. Aspects of *in situ*, *in vitro* germination and mycorrhizal partners of *Liparis loeselii*. Acta Biol. Szegediensis 49: 137-139.

Jeanes, J.A. & G.N. Backhouse. 2000. Pictorial Guide to Wild Orchids of Victoria Australia. Zoonectics, Seaford, Victoria.

Perkins, A.J. & P.A. McGee. 1995. Distribution of the orchid mycorrhizal fungus, *Rhizoctonia solani*, in relation to its host, *Pterostylis acuminata*, in the field. Austral. J. Bot. 43: 565-575.

Rasmussen, H.N., T.F. Andersen, T.F. & B. Johansen. 1990. Temperature sensitivity of *in vitro* germination and seedling development of *Dactylorhiza majalis* (Orchidaceae) with and without a mycorrhizal fungus. Pl. Cell Envir. 13: 171-177.

Rasmussen, H.N. & D.F. Whigham. 1993. Seed ecology of dust seeds *in situ:* a new study technique and it application in terrestrial orchids. Amer J. Bot. 80: 1374-1378.

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SYMBIOTIC GERMINATION OF THREATENED AUSTRALIAN TERRESTRIAL ORCHIDS AND THE EFFECT OF NURSERY POTTING MEDIA ON SEEDLING SURVIVAL

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KEY WORDS: symbiotic propagation, endangered orchids, potting media

The *ex situ* propagation program at the Royal Botanic Gardens, Melbourne (RBG)

Since the early 1990s, the RBG has contributed to the conservation of Victoria's Endangered orchids through its *ex situ* propagation program. Working cooperatively with the Victorian Department of Sustainability and Environment (DSE), the Melbourne Zoo, the Australasian Native Orchid Society, The University of Melbourne, RMIT University and Parks Victoria, research and development has lead to a greater understanding of Victoria's terrestrial orchids and their associated mycorrhizal fungi, and assisted in the implementation of Recovery Plans. Victoria has high orchid biodiversity, with nearly a quarter of Australia's 1250 species occurring in only 3% percent of the land area. Many of Victoria's orchid species are under threat of extinction, and with nearly 40% endemism (Backhouse and Cameron 2005) this is of national and even global concern.

The aim of the *ex situ* propagation program at the RBG is to provide symbiotically grown orchids for reintroduction into natural habitats. Plants are also being propagated to establish *ex situ* populations of species that are under high risk of extinction. *Ex situ* populations provide seed orchards that decrease the pressure of seed collection on threatened wild populations. Currently, for over 45 threatened orchid species from the genera *Caladenia, Diuris, Thelymitra, Paracaleana, Prasophyllum, Pterostylis, Calochilus* and *Corunastylis*, isolation of mycorrhizal fungi and symbiotic germination of seed are being attempted. To date, these techniques have been successful for 27 species belonging to the genera *Caladenia, Diuris, Thelymitra* and *Pterostylis*. Laboratory-grown seedlings from these genera have been acclimatised to the nursery environment. The step involving seedling transfer to nursery media has regularly resulted in seedling losses, being particularly noticeable after the first dormancy period. Apart from providing appropriate environmental conditions such as humidity, light and aeration, the design of the nursery-potting medium was considered to be of importance to ensure that adequate moisture levels are maintained with minimal risk of tuber death in overly wet substrates.

Potting media

The effect of four different nursery media on seedling survival was investigated for a range of species from the genera *Caladenia, Diuris, Thelymitra* and *Pterostylis* and their emergence after summer dormancy was recorded. The potting media used included: the RBG cutting mix, the Australasian Native Orchid Society (ANOS) mix, a Zoo mix equivalent and our own pine-bark based mix. The RBG cutting mix is a highly aerated gravel based mix routinely used at the in RBG nursery for propagating plants from cuttings. The ANOS mix was developed for the cultivation of Australian terrestrial orchids and is high in naturally occurring organic matter including bark, leaf mould and litter. The Zoo mix is used by the Melbourne Zoo in their *ex situ* terrestrial orchid collection and our equivalent comprises of a

1:1 ratio of perlite and a Debco potting mix (composted pine bark). Our pine-bark based mix contained the Debco potting mix with the course and fine particles removed by sieving, which was mixed three parts to one part perlite.

All species were germinated with mycorrhizal fungi isolated from the same population as the seed was collected from, according to methods described by Clements *et al.* (1986). In 2005 symbiotically germinated seedlings were transferred into these four mixes in Hykos® (40 celled trays). As seedlings were being grown primarily for conservation purposes they were transferred after reaching a size where *ex vitro* survival was deemed possible. This meant that the sample size of the different species varied and all species were transferred over a number of months. Those species with sufficient sample sizes to compare their survival in all four media were from the genera *Caladenia* and *Diuris* and included: *C. amoena* D.L. Jones, *C. calcicola* G.W. Carr, *C. cruciformis* D.L. Jones, *C. robinsonii* G.W. Carr, *C. versicolor* G.W. Carr, *C. xanthochila* D. Beardsell & C. Beardsell, *D. fragrantissima* D.L. Jones & M.A. Clem. and *D.* sp. aff. *chryseopsis* (Basalt Plains) sensu Ross & Walsh (2003).

Survival results

The survival (emergence after summer dormancy) of these species was recorded in July 2006. All of the *Caladenia* species had their highest survival in either the Zoo mix equivalent or our pine-bark based mix and many had little or no survival in the ANOS and RBG cutting mixes. Two species had reasonable survival in all four mixes, C. *xanthochila* (with more than 50%) and *C. calcicola* (with more than 30%). Overall the *Diuris* species had lower survival than the *Caladenia* species. These results are unusual as *Caladenia* species are generally recognised to be more difficult to grow than *Diuris* species (Richards *et al.* 1984). The *Diuris* species had similar survival in the Zoo equivalent mix, our pine-bark based mix and RBG cutting mix (15-30%). Their survival in the ANOS mix differed, as the highest survival for *D.* sp. aff. *chryseopsis* (40%) was observed in this mix and the lowest for *D. fragrantissima* (7%). *D.* sp. aff. *chryseopsis* was the only species of the eight tested to achieve highest survival rates in the ANOS mix. The low survival of the *Diuris* species is unlikely to be due to the range of potting mixes used. The Zoo mix is primarily used for the transferal of asymbiotically germinated *D. fragrantissima* seedlings at the Melbourne Zoo *ex situ* collection. These plants are transferred to potting media after tuber formation, at approximately 18-months old, and survival rates of up to 90% are regularly observed (R. Thomson pers. obs. 2006). The smaller size of the symbiotic *Diuris* seedlings transferred in 2005 may have lead to below average survival. In 2006, we grew *Diuris* seedling in the *in vitro* environment until they were larger before transferal to the nursery environment. Both *Pterostylis* and *Thelymitra* species transferred into the Zoo mix equivalent and our pine-bark based mix survived the summer dormancy. These mixes appeared to be the most appropriate mixes for the range of species and genera tested.

Implications for potting mix selection

Survival results for the *Caladenia* and *Diuris* species lead to the selection of the Zoo mix equivalent and our pine-bark based mix for the use in further transferal of symbiotic seedlings. These two mixes where used exclusively in seedling transferals conducted during 2006. In addition to providing maximum plant survival rates, the mixes are both replicable; as both the Debco potting mix and the perlite used in these mixes are quality tested and conform to Australian Standards. The lack of replicable components in the ANOS mix may explain why many of the species tested had such low survival in this mix when it is routinely used with success by ANOS members. The ANOS mix has several components collected from the natural environment and these components differ depending on where they are collected. The mix we used was quite dry and water repellent. It is possible that if we used an ANOS mix made with components from a different source we would have observed different results.

Overall program outcome

The RBG's *ex situ* propagation program has produced hundreds of plants of a number of endangered species for reintroduction and *ex situ* collections.

Some of these plants have larger leaves than adult plants of the same species observed in the field. Many *Caladenia* species flowered in the nursery 18 months after germination including *C. amoena, C. xanthochila, C. hastata, C. calcicola* and *C. robinsonii*. Reintroductions of two *Pterostylis* species propagated were undertaken by DSE staff in 2006. The majority of remaining plants will be grown on in the nursery and reintroduction conducted when climatic conditions are conducive to survival *in situ*.

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LITERATURE CITED

Backhouse, G. & D. Cameron. 2005. Application of the IUCN 2001 Red List categories in determining the conservation status of native orchids of Victoria, Australia. Selbyana 26: 55-74.

Clements, M., P.J. Cribb & H. Muir. 1986. A preliminary report on the symbiotic germination of European terrestrial orchids. Kew Bull. 14: 437-445.

Richards, H., R. Wotton & R. Datodi. 1984. *In*: Cultivation of Australian native orchids. Australian Native Orchid Society Victoria Group Inc.

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THE ORCHID RECOVERY PROGRAM AT ILLINOIS COLLEGE – A SUCCESSFUL BLEND OF TEACHING, RESEARCH AND UNDERGRADUATE STUDENT PARTICIPATION TO BENEFIT ORCHID CONSERVATION

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KEY WORDS: orchid conservation, North America, undergraduate students, research, liberal arts

A decade ago (1996), the Orchid Recovery Program was established at Illinois College – a private liberal arts college – to promote the conservation of rare orchids through propagation. The school's rural location, small size (1,000+ undergraduate students), and heavy teaching load (= 12 hours/semester) posed serious challenges to the establishment of this research program. Nevertheless, over the years several aspects of the College were identified, targeted, and integrated to successfully blend the student academic experience with orchid conservation. This paper provides a summary of the mechanisms that led to the establishment and success of the program, and describes how meaningful research is indeed possible at smaller colleges, not just at large research institutions.

Historically, large research institutions in the United States and elsewhere have instilled an attitude of "publish or perish" as a necessary means to achieve tenure and promotion in its faculty members. During the past 10-20 years, faculty members have also faced increasing pressure to secure sizable external grants to support research and, in some cases, salary. As a result, faculty members appear to be evolving into aggressive entrepreneurs, raising concerns that such a mindset has the potential to adversely affect science itself. This shift has also led to a kind of natural selection where "high tech" research is favored over basic organismal biology, probably because the former brings in more cash flow and prestige. Compounding matters, specialists trained in organismal biology are being steadily phased out - usually via retirement - in favor of the entrepreneur. Those that remain at the larger institutions have been relegated to smaller working spaces (*e.g.*, hallway closets), or become employed elsewhere. Some find shelter from administrators by teaching. Ironically, these organismal biologists are still in demand on some levels, including those that cut and grind specimens in search of high tech answers.

This environmental pressure has triggered an evolutionary process in academia and a significant transformation is now underway – young organismal biologists are seeking employment at smaller, teaching oriented, undergraduate institutions where the pressure to publish and secure external funds is (currently) significantly less. Nevertheless, administrators at smaller institutions now recognize the importance of undergraduate research, probably because of its potential to lure exceptional prospective students (= cash) away from competing institutions. This seems especially true of private colleges whose livelihood depends on student enrollment and retention. Consequently, the expectation for undergraduate research in this setting is on the rise, and faculty members are faced with the added burden of also maintaining high teaching standards.

The abovementioned scenario seems dire for faculty, students and science itself. When the ongoing global loss of biological diversity is also considered, the future is clearly troublesome. The next generation – today's students – will inherit a wildly changing planet and they will also serve as caretakers of the world's natural resources. To do the latter effectively, they must truly care about all creatures great and small, and they must understand and appreciate how each species interacts within the broader ecological framework. Unfortunately, the music of life itself has been masked or replaced by the sounds produced by

our machines, and poisoned by the pollution that made these machines. Technological devices (*e.g.*, cell phones, video games) have infiltrated society and are firmly embedded within the everyday lives of today's college student. Technology is inevitable and good, but only if used responsibly, and not at the expense of a quality education, or at the expense of the environment.

THE FOUNDATION FOR ORCHID RESEARCH AT A SMALL COLLEGE

Founded in 1829, Illinois College was the first college or university in the state of Illinois to hold classes and award higher education degrees. Today, the College educates *ca*. 1,000 undergraduates in a curriculum rooted in the liberal arts. The Mission Statement of the College emphasizes the "development of mind and character needed for fulfilling lives of leadership and service". Similarly, the College's Statement of Community Responsibility emphasizes that "we are all caretakers of our community and recognize that our individual responsibilities are essential for nurturing collaborative relationships, critical exploration, and global awareness". Thus, the public doctrine of the College is structured, at least in theory, to support biological (orchid) conservation. The fusion of orchid research into the curriculum, therefore, can be viewed as one tool by which undergraduate students become liberally educated and ultimately, responsible caretakers. The Biology Department at Illinois College has catalyzed this process even further. Biology students (= science majors) are provided with introductory courses their freshman year (*e.g.*, basic chemistry and biology), then have the opportunity to take specialized courses in several tracts of study (*e.g.*, marine biology, botany, pre-medicine).

One such course in particular, Introduction to Research, has been instrumental in engaging undergraduates in research and the scientific method. As a regularly offered course, it is counted towards the faculty member's teaching load. Therefore, it serves to ease the burden of a high teaching load normally associated with smaller colleges. A prerequisite of the course are two vigorous core courses required of science majors their freshmen year, General Biology I and II. Students that then enroll in Introduction to Research are adequately prepared to conduct experiments because they have received fundamental biological knowledge beforehand. Once enrolled, students learn about research by doing research.

According to student evaluations and interviews, a key ingredient in the success of the Introduction to Research course lies in the nature of the experiments, their outcomes, and their application to real-world problems. Students that design and conduct experiments aimed at improving orchid conservation are provided with a greater sense of responsibility and personal achievement. Students are often awestruck by the realization that they are actually engaged in a research project that has the potential to ultimately save an orchid species from extinction. For most, this serves as a "wake up call" that opens their minds and hearts to the plight of orchids and other organisms threatened worldwide. Many also realize the power of the scientific method, and realize that science is both challenging, but enjoyable.

THE COMPONENTS OF THE ORCHID RECOVERY PROGRAM AT ILLINOIS COLLEGE.

Upon completion of the Introduction to Research course, students interested in pursuing other, similar projects are encouraged to do so during their last two years of study. This was the catalyst for the formation of Illinois College's Orchid Recovery Program. The core of the Orchid Recovery Program lies in the undergraduates that fuel it. Students play an active role collecting information (literature searches), designing experiments, writing grants (internal and external to the College), gathering/analyzing data, contributing to manuscripts, and giving scientific presentations. For this to work effectively, the personality traits of each student must be identified so that his or her strengths can be realized. Thus, each student is viewed as a unique, pliable individual with a different set of strengths – not a clone of the supervisor. Matching the various strengths and personality traits within the lab group is challenging but possible considering that each student was previously evaluated via the teaching process (General Biology I, II and Introduction to Research). This is yet another way that teaching complements research. Because of the College's small size and (still) healthy endowment (>$100 million), a manageable number of students (*e.g.*, 1-5) are engaged in the research program at any given time, and some receive pay via college-supported stipends.

Students with seniority and/or the most experience

serve as leaders within the group, and are provided with personal desk space within the lab. As a result, student leaders serve as a conduit between the supervisor and the students with the least experience. This is a critical aspect of the program because the student leader's role makes it possible for the supervisor to concentrate more on teaching nine months of the year (September-May). The other three months (June-August) are dedicated entirely to research. During this time, the supervisor works closely with each student to design/implement experiments, improve training, travel, and carry out field-based research (*e.g.*, seedling reintroductions). Students also collaborate directly with leading specialists at other institutions during this time. Once this fundamental groundwork has been established, the supervisor is then free to concentrate on teaching once again. In short, students are trained over the summer, and collect data when classes are in session, often between classes. Data collected during the teaching months by students are then analyzed over the next summer, leading to manuscripts and presentations. Because students play an integral role in this process, they often (deservingly) serve as co-authors. The end result is a liberally educated, self-confident, responsible, and experienced graduate (= caretaker) that is marketable through the eyes of many graduate programs nationwide. Some students have opted to continue research aimed at orchid conservation (*e.g.*, Kurt Piskin, PhD student, St. Louis University; Scott Stewart, PhD student, University of Florida), whereas others have chosen other fields (*e.g.*, Chris Wagoner, medical student, University of Illinois). Regardless of their ultimate career choice, the Orchid Recovery Program has instilled in each student an appreciation for orchids and their conservation (see Stewart 2002, and Zettler 2006).

Specific Examples of Student Research Projects via the Orchid Recovery Program

During the past 10 years, a total of 36 undergraduate students have received training through the Orchid Recovery Program. Among the rare orchid taxa studied include two U.S. Federal listed *Platanthera* species, as well as several state-listed species in other genera (Table 1). Most of the research has investigated the role and use of mycorrhizal fungi to propagate terrestrial orchids from seed (= symbiotic seed germination). Several species have been successfully cultivated in this manner leading to seedling reintroduction in suitable habitats (*e.g.*, *Habenaria macroceratitis* Willd., *Spiranthes brevilabris* Lindl.). Recently (2004-present), students have examined the feasibility of applying the symbiotic technique to epiphytic orchids using *Epidendrum nocturnum* Jacquin as a model. Such student-led work has the potential to change and improve the way epiphytic orchids are conserved worldwide. Ongoing and future work is also being carried out to improve seedling survival in *Platanthera holochila* (Hbd.) Krzl. - a U.S. Federal listed (global rank C1)

TABLE 1. Endangered, rare or otherwise uncommon orchids studied by undergraduate students via the Orchid Recovery Program at Illinois College during the past decade. All references listed were authored or co-authored by one or more undergraduate students.

Orchid	Status	Reference(s)
Epidendrum nocturnum (Jacq.)	Endangered in FL	Zettler *et al.* 2006
Habenaria macroceratitis Willd.	Endangered in FL	Stewart & Zettler, 2002
Platanthera holochila (Hbd.) Krzl.	U.S. Federal Endangered	Zettler *et al.* 2005a
P. integra (Nutt.) Lindl.	Endangered in FL, NJ, TN	Zettler *et al.* 2000
P. integrilabia (Correll) Luer	U.S. Federal Threatened (proposed)	Yoder *et al.* 2000
P. leucophaea (Nutt.) Lindl.	U.S. Federal Threatened	Bowles *et al.* 2002 Zettler *et al.* 2001 Zettler *et al.* 2005b
Spiranthes brevilabris Lindl.	Endangered in FL	Stewart *et al.* 2003

Hawaiian endemic – in collaboration with Steve Perlman (National Tropical Botanical Garden). Another significant, student-led project was recently initiated that aims to propagate/reintroduce four rare orchids of south Florida: *Epidendrum amphistomum* A. Richard, *E. rigidum* Jacq., *Polystachya concreta* (Jacquin) Garay & Sweet and *Vanilla phaeantha* Rchb.f. Students working in collaboration with Scott Stewart (University of Florida) and Larry Richardson (U.S. Fish & Wildlife Service) are attempting to cultivate these orchids with and without fungi. So far, at least two of the species have been propagated to the leaf-bearing stage *in vitro*.

Overall, research in the Orchid Recovery Program has led to 12 published papers in scientific (refereed) journals co-authored by 13 different undergraduate students. In addition, 20 different students have co-authored 20 published abstracts via presentations at national or international meetings. This program may, therefore, be viewed as a successful 10-year experiment to promote orchid conservation in North America. In doing so, it has instilled an appreciation for biological diversity in the next generation of caretakers. It also represents one working model of how research can be established and maintained at similar small colleges in the United States and perhaps elsewhere. It is this author's opinion that conservation must infiltrate (expand) within academia, in both vocabulary and practice. It seems unfortunate that a disproportionate number of organismal biologists, at large and small institutions alike, have little or no (direct) link to conservation. If true, such apathy or indifference towards our planet's natural resources is especially disheartening when these academicians themselves should be at the forefront of conservation. Unless this perceived attitude changes, the species extinctions projected to occur this century will almost certainly be realized.

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LITERATURE CITED

Bowles, M.L., K.A. Jacobs, L.W. Zettler &T. Wilson Delaney. 2002. Crossing effects on seed viability and experimental germination of the Federal threatened eastern prairie fringed orchid, *Platanthera leucophaea* (Nutt.) Lindl. Rhodora 104: 14-30.

Stewart, S.L. 2002. Saving a native orchid. Orchids Magazine 71: 916-919.

Stewart, S.L. & L.W. Zettler. 2002. Symbiotic germination of three semi-aquatic rein orchids (*Habenaria macroceratitis*, *H. quinqueseta*, *H. repens*) from Florida. Aquatic Bot. 72: 25-35.

Yoder, J.A., S.L. Stewart & L.W. Zettler. 2000. Water requirements of terrestrial andepiphytic orchid seeds and seedlings, and evidence for water uptake by means of mycotrophy. Plant Sci. 156: 145-150.

Zettler, L.W. 2006. Endemics: ghosts in the making. Native Orchid Conf. J. 3(3): 14-17.

Zettler, L.W., S. Perlman, D.J. Dennis, S.E. Hopkins & S.B. Poulter. 2005. Symbiotic germination of the Federally endangered Hawaiian endemic, *Platanthera holochila* (Orchidaceae) using a mycobiont from Florida: a conservation dilemma. Selbyana (1,2): 269-276.

Zettler, L.W., K.A. Piskin, S.L. Stewart, J.J. Hartsock, M.L. Bowles & T.J. Bell. 2005. Protocorm mycobionts of a Federally threatened orchid, *Platanthera leucophaea*, and a technique to promote leaf formation in seedlings. Stud. Mycol. 53: 153-161.

Zettler, L.W., S.B. Poulter, K.I. McDonald & S.L. Stewart. 2007. Conservation-driven propagation of an epiphytic orchid (*Epidendrum nocturnum*) with a mycorrhizal fungus. HortSci. 42: (in press).

Zettler, L.W., S.L. Stewart, M.L. Bowles & K.A. Jacobs. 2001. Mycorrhizal fungi and cold-assisted symbiotic germination of the Federally threatened eastern prairie fringed orchid, *Platanthera leucophaea* (Nutt.) Lindl. Am. Mid. Nat. 145: 168-175.

Zettler, L.W., J.A. Sunley & T. Wilson Delaney. 2000. Symbiotic seed germination ofan orchid in decline (*Platanthera integra*) from the Green Swamp, North Carolina. Castanea 65: 207-212.

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2ND INTERNATIONAL CONFERENCE ON NEOTROPICAL ORCHIDOLOGY

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THE ORCHIDACEAE OF "PARQUE MUNICIPAL DE MUCUGE", BAHIA, BRAZIL

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PALABRAS CLAVE: campo rupestre, Chapada Diamantina, orchids, taxonomy

Introduction

The Orchidaceae are one of the largest families of flowering plants, with around 20.000 species in approximately 850 genera (Atwood 1986, Dressler 1993). It is well represented in Brazil, with about 2.350 species and 200 genera (Pabst & Dungs 1975, 1977). In Bahia were registered 285 species (Pabst & Dungs 1975, 1977, Harley & Mayo 1980, Harley & Simmons 1986, Toscano-de-Brito 1995, 1998, Toscano-de-Brito & Queiroz 2003).

The Espinhaço chain is composed by two main regions, one in Minas Gerais and the other in Bahia. The last is known as Chapada Diamantina. In its southern portion, the Chapada Diamantina splits into two separate chains, with the Serra do Rio de Contas and the Serra das Almas in the west, and the Serra do Sincorá in the east (Harley 1995). The Chapada Diamantina survey registered the presence of 139 species of Orchidaceae in this region (Harley & Simmons 1986, Toscano-de-Brito 1995, 1998, Toscano-de-Brito & Queiroz 2003).

The Parque Municipal de Mucugê (PMM) is located at the municipality of Mucugê, at Serra do Sincorá, around 4 km of the Mucugê town, at 12° 59'02"-13°00'18"S and 41°19'40"-41°21'33"W, at an altitude of 1.000 m above sea level, and occupies an area of 4.5 km^2. The predominant vegetation is the "campos rupestres", which is an important center of diversity of the Brazilian flora, with a large number of endemic species (Harley & Simmons 1986, Harley 1995). Orchidaceae presents a high floristic importance, being always between the ten largest families in species richness (Harley & Simmons 1986, Giulietti et al. 1987, Stannard 1995, Guedes & Orge 1998, Pirani et al. 2003, Zappi et al. 2003).

The present work aimed at providing a detailed survey of the Orchidaceae in the Parque Municipal de Mucugê, as well as descriptions, illustrations and identification key.

Materials and Methods

Specimen was carried out trough monthly sampling during 12 months, between June 2002 and May 2003. The specimens were deposited at the Universidade Estadual de Feira de Santana herbarium (HUEFS). The ALCB, CEPEC, HRB, HUEFS, K, SPF and W collection were consulted.

Results and Discussion

Currently, 22 genera and 35 species of orchids were found at the Parque Municipal de Mucugê (Tab. 1), of which one is a natural hybrid (Azevedo *et al*., 2006). The most representative genera are *Bulbophyllum* Thou. (3 spp. e 1 nothosp.), *Epidendrum* L. (4 spp.), *Octomeria* R.Br. (3 spp.) and *Prescottia* Lindl. (3 spp.). Of the remaining, 68 % of the genera present only one species.

Some of the orchid species found at the PMM present wide geographical distribution, being found in other countries, and well distributed in Brazil. From the 35 species colleted at PMM, four are new records for the Bahia State, mainly known previously from Southern and Southeastern Brazil. Eight are new records for the Chapada Diamantina region and another 18 are new records for the municipality of Mucugê. The area presents some species endemic to

TABLE 1. List of Orchidaceae at the Parque Municipal de Mucugê.

Species	Species
Acianthera hamosa (Barb.Rodr.) Pridgeon & M.W.Chase	*Epidendrum secundum* Jacq.
Acianthera ochreata (Lindl.) Pridgeon & M.W.Chase	*Epidendrum warasii* Pabst
subsp. *ochreata*	*Epistephium lucidum* Cogn.
Anathallis microphyta (Barb.Rodr.) C.O.Azevedo & Van den Berg	*Habenaria fluminensis* Hoehne
Anathallis montipelladensis (Hoehne) F.Barros	*Maxillaria notylioglossa* Rchb.f.
Brassavola tuberculata Hook.	*Octomeria alexandrii* Schltr.
Bulbophyllum cribbianum Toscano	*Octomeria flabellifera* Pabst
Bulbophyllum involutum Borba, Semir & F.Barros	*Octomeria sagittata* (Rchb.f.) Garay
Bulbophyllum weddellii (Lindl.) Rchb.f.	*Oncidium blanchetii* Rchb.f.
Bulbophyllum ?cipoense Borba & Semir	*Polystachya micrantha* Schltr.
Campylocentrum micranthum (Lindl.) Rolfe	*Prescottia leptostachya* Lindl.
Cattleya elongata Barb.Rodr	*Prescottia montana* Barb.Rodr.
Cleistes exilis Hoehne	*Prescottia stachyodes* (Sw.) Lindl.
Cyrtopodium aliciae L.Linden & Rolfe	*Prosthechea moojenii* (Pabst) W.E.Higgins
Cyrtopodium polyphyllum (Vell.) Pabst ex F.Barros	*Scaphyglottis modesta* Schltr.
Encyclia alboxanthina Fowlie	*Sobralia sessilis* Lindl.
Epidendrum cristatum Ruiz & Pav.	*Sophronitis bahiensis* (Schltr.) Van den Berg & M.W.Chase
Epidendrum orchidiflorum Salzm. ex Lindl.	*Thelyschista ghillanyi* (Pabst) Garay

the Chapada Diamantina such as *Encyclia alboxanthina* Fowlie, *Sophronitis bahiensis* (Schltr.) Van den Berg & M.W.Chase and *Thelyschista ghillanyi* (Pabst) Garay.

LITERATURE CITED

Atwood, J. 1986. The size of the Orchidaceae and the systematic distribution of epiphytic orchids. Selbyana 9: 171-186.

Azevedo, C.O., E.L.Borba & C. van den Berg. 2006. Evidence from allozyme markers for natural hybridization and introgression in *Bulbophyllum involutum* and *B. weddellii* (Orchidaceae). Revista Brasileira de Botânica; Publicação Oficial da Sociedade Botânica do Brasil, Regional de São Paulo.

Cribb, P. & A.L.V. Toscano-de-Brito. 1996. Histórico. *In*: S. Sprunger, P. Cribb & A.L.V. Toscano-de-Brito (eds.) Iconographie des Orchidées du Brèsil. vol. 1. Reinhardt, Basle, pp. 42-47.

Dressler, L.R. 1993. Phylogeny and classification of the orchid Family. Dioscorides Press, Portland, 314 p.

Giulietti, A.M., N.L. Menezes; J.R. Pirani; M. Meguro & M.G.L. Wanderley. 1987. Flora da Serra do Cipó, Minas Gerais: caracterização e lista de espécies. Boletim de Botânica. Departamento de Botânica. Instituto de Biociências. Universidade de São Paulo 9: 1-151.

Guedes, M.L.S. & M.D. Orge (eds.). 1998. Checklist das espécies vasculares do Morro do Pai Inácio (Palmeiras) e Serra da Chapadinha (Lençóis), Chapada Diamantina, Bahia, Brasil. UFBA, Salvador, 68 p.

Harley, R.M. 1995. Introdução. *In*: B.L. Stannard (ed.) Flora of the Pico das Almas: Chapada Diamantina, Bahia, Brazil. Royal Botanic Gardens, London, pp. 43-76.

Harley, R.M. & S.J. Mayo. 1980. Towards a Checklist of the Flora of Bahia. Royal Botanic Gardens, London, 245 p.

Harley, R.M. & N.A. Simmons. 1986. Florula of Mucugê: Chapada Diamantina - Bahia, Brazil. Royal Botanic Gardens, London, 228 p.

Pabst, G.F.J. & F. Dungs. 1975. Orchidaceae Brasilienses. vol. 1. Kurt Schmersow, Hildesheim, 408 p.

Pabst, G.F.J. & F. Dungs. 1977. Orchidaceae Brasilienses. vol. 2. Kurt Schmersow, Hildesheim, 418 p.

Pirani, J.R., R. Mello-Silva & A.M. Giulietti. 2003. Flora de Grão-Mogol, Minas Gerais, Brasil. Boletim de Botânica. Departamento de Botânica. Instituto de

Biociências. Universidade de São Paulo 21(1): 1-24.

Sprunger, S., P. Cribb & A.L.V. Toscano-de-Brito (eds.) 1996. Iconographie des Orchidées du Brèsil. vol. 1. Reinhardt, Basle. 540 p. il.

Stannard, B.L. (ed.) 1995. Flora of the Pico das Almas: Chapada Diamantina, Bahia, Brazil. Royal Botanic Gardens, London. 853 p.

Toscano-de-Brito, A.L.V. 1995. Orchidaceae. *In:* B. L. Stannard (ed.) Flora of the Pico das Almas: Chapada Diamantina, Bahia, Brazil. Royal Botanic Gardens, London, pp. 725-767.

Toscano-de-Brito, A.L.V. 1998. Orchidaceae. *In:* M.L.S. Guedes & M.D. Orge (eds.) Checklist das Espécies Vasculares do Morro do Pai Inácio (Palmeiras) e Serra da Chapadinha (Lençóis), Chapada Diamantina, Bahia, Brasil. UFBA, Salvador, pp. 53-54.

Toscano-de-Brito, A.L.V. & L.P. Queiroz. 2003. Orchidaceae. *In*: D.C. Zappi, E. Lucas, B.L. Stannard, E.N. Lughadha, J.R. Pirani, L.P. Queiroz, S. Atkins, D.J.N. Hind, A.M. Giulietti, R.M. Harley & A.M. Carvalho. Lista das plantas vasculares de Catolés, Chapada Diamantina, Bahia, Brasil. Boletim de Botânica. Departamento de Botânica. Instituto de Biociências. Universidade de São Paulo 21(2): 396-397.

E. Lucas, B.L. Stannard, E.N. Lughadha, J.R. Pirani, L.P. Queiroz, S. Atkins, D.J.N. Hind, A.M. Giulietti, R.M. Harley & A.M. Carvalho. 2003. Lista das plantas vasculares de Catolés, Chapada Diamantina, Bahia, Brasil. Boletim de Botânica. Departamento de Botânica. Instituto de Biociências. Universidade de São Paulo 21(2): 345-398.

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LAS ORQUÍDEAS DEL PARQUE NACIONAL BARRA HONDA, GUANACASTE, COSTA RICA

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PALABRAS CLAVE: áreas protegidas, Costa Rica, estudios florísticos, Orchidaceae, Parque Nacional Barra Honda

Introducción

Los trabajos florísticos constituyen fuentes de información de suma importancia pues entre otros aspectos, determinan el número de especies presentes en una región y permiten su correcta identificación (Atwood 1987, Pupulin 1998). La carencia de este tipo de trabajos dificulta futuras investigaciones en los diversos planos de la biología, la conservación y la educación ambiental. Costa Rica posee uno de los sistemas de áreas de conservación más importantes a nivel mundial. Cerca del 25% del territorio se encuentra protegido en unas 155 áreas silvestres, reservas biológicas y parques nacionales (Boza 1986). A pesar de esto, el aporte de los estudios florísticos al conocimiento de las especies de orquídeas que habitan en dichas áreas ha sido escazo y los inventarios respaldados por especímenes en herbarios son una excepción (Atwood 1987, Pupulin 1998, Gómez-Laurito & R. Ortiz 2004). La mayoría de las áreas protegidas en Costa Rica carecen de esta información, la cual se encuentra muchas veces dispersa en registros de herbarios locales o extranjeros (Jiménez & Grayum 2002, Pupulin 2003).

Materiales y métodos

El Parque Nacional Barra Honda (PNBH) se ubica en el cantón de Nicoya, provincia de Guanacaste (Boza 1986) (fig. 1). El interés de crear este parque surgió como una medida para proteger el sistema de cavernas de la región de Nicoya, originado por los eventos geológicos desde el Paleoceno Superior y Eoceno Inferior hasta el presente. Esta zona representa un área geológica muy importante para el país. (Mora 1978, Aguilar & Denyer 2001, Jaccard *et al.* 2001). No obstante, la proteción de los recursos biológicos e hídricos es también otro de los objetivos se su creación. El PNBH comprende una extensión de 2.295 hectáreas donde predomina el bosque seco semideciduo del Pacífico Norte, caracterizado por una marcada estacionalidad climática (Tosi 1969, Janzen 1983). Históricamente, estos bosques han sufrido una fuerte intervención humana producto de la deforestación y el desarrollo de las actividades agropecuarias. Por otro lado, han sido bosques poco atractivos para los orquideólogos dada su baja diversidad de orquídeas en comparación con los bosques húmedos montanos y premontanos. Ante la fragilidad de estos ecosistemas de bosque seco, únicos en el país y la carencia de datos que permitan evaluar el número de especies y su identidad, es indispensable el aporte de los estudios florísticos para llevar a cabo acciones de conservación prioritarias en esta área (Boza 1986, Janzen 1983).

El presente estudio se realiza con base en recolectas de orquídeas en los diferentes sitios del PNBH. Durante el mes de Julio del 2005 y Febrero del 2006 se obtuvieron los datos que aquí se presentan. El material recolectado fue llevado a cultivo en las colecciones vivas del Jardín Botánico Lankester, Universidad de Costa Rica. Los testigos fueron depositados en las colecciones en alcohol del Jardín Botánico Lankester (JBL) y en el Herbario Nacional de Costa Rica (CR).

Resultados

En la primera etapa del estudio se determinó que la flora de orquídeas está compuesta por 26 especies en 23 géneros. Los géneros más representados son *Epidendrun* (3 especies), *Scaphyglottis* y *Specklinia* (ambos con 2 especies). Los demás

FIGURA 1. Ubicación del Parque Nacional Barra Honda.

géneros (88.5%) están respresentados en el parque por una sola especie. El 73% de las especies son epífitas y un 27 % presenta un hábito terrestre (cuadro 1). El período de floración se concentra entre los meses de Noviembre a Abril, básicamente durante la estación seca.

Discusión

Algunas de las especies del PNBH tienen una amplia distribución a nivel nacional y habitan en toda la costa Pacífica, tanto en las zonas húmedas del sur como en las áreas secas estacionales del norte y parte del Valle Central. Estas especies son: *Epidendrum coronatum* Ruiz & Pav., *Epidendrum stamfordianum* Bateman, *Epidendrum vulgoamparoanum* Hágsater y *Scaphyglottis stellata* Lodd. ex Lindl. Tres especies, *Pleurothallis quadrifida* (Lex.) Lindl., *Scaphyglottis micrantha* (Lindl.) Ames & Correll y *Sobralia decora* Bateman presentan este tipo de distribución pero se les puede encontrar también en la parte húmeda del Caribe norte. Además, *Brassavola nodosa* (L.) Lindl., *Trigonidium egertonianum* Bateman ex Lindl., *Catasetum maculatum* Kunth, *Specklinia grobyi* (Bateman ex Lindl.) F. Barros y *Dimerandra emarginata* (G.Mey.) Hoehne, pueden distribuirse, tanto a lo largo de toda la vertiente Pacífica como en la vertiente Caribe. En el PNBH estas especies se pueden observar con relativa frecuencia en los bosques secundarios poco alterados.

Dentro de la región biogeográfica del Pacífico Norte, existe otro grupo de especies que se encuentran en las zonas estacionales húmedas y secas hacia el norte de la cuenca del río Grande de Tárcoles y alcanzan sitios estacionales cercanos al Valle Central. Este grupo presenta su límite de distribución sureño en las áreas circunvecinas al Cerro Turrubares, la cuenca del Tárcoles y el río Candelaria y su hábitat corresponde más con los bosques estacionales secos del norte (Jiménez & Grayum 2002). Dentro de este grupo encontramos *Barkeria obovata* (C. Presl) Christenson, *Cohniella cebolleta* (Jacq.) Christenson, *Cyrtopodium paniculatum* (Ruiz & Pav.) Garay, *Encyclia cordigera* (Kunth) Dressler, *Laelia rubescens* Lindl. y *Trichosalpinx blaisdellii* (S. Watson) Luer. Estos datos son apoyados por estudios florísticos en la región central y sur de la costa Pacífica, donde no se reporta la presencia de estas especies (Pupulin 1998, Weber *et. al* 2001, Jiménez & Grayum 2002). Sin embargo, esta misma área biogeográfica es el límite para algunas especies del Pacífico Central y Sur que crecen en las áreas más humedas y poco estacionales y no alcanzan una distribución más norteña. Este patrón es observado en la flora de orquídeas del PNBH y especies como *Specklinia corniculata* (Sw.) Steud., *Aspasia epidendroides* Lindl., *Ionopsis satyrioides* (Sw.) Rchb.f., *Trizeuxis falcata* Lindl., *Prosthechea abbreviata* (Schltr.) W.E.Higgins, *Campylocentrum multiflorum* Schltr. y *C. micranthum* Lindl., entre otras, se distribuyen al sur de la costa Pacífica sin alcanzar las áreas estacionales del Pacífico Norte (Pupulin 1998, Jiménez & Grayum 2002).

En el PNBH se protege la flor nacional, *Guarianthe skinneri* (Bateman) Dressler & W.E. Higgins. Su distribución es también amplia a lo largo del territorio nacional, sin embargo sus poblaciones silvestres han sido reducidas por la deforestación y excesiva recolecta. Algunas poblaciones se encuentran en el PNBH sin embargo es difícil obsevarlas aparentemente por la extracción ilegal dentro del parque.

Siete especies con hábito terrestre se encuentran en el parque. Durante el período seco, todas las plantas de estas especies presentan un comportamiento deciduo, perdiendo sus hojas durante los

CUADRO 1. Hábito y distribución general de las orquídeas del Parque Nacional Barra Honda.

Género y especie	Hábito	Amplia distribución	Influencia Pacífica	Pacífico Norte
1. *Barkeria obovata* (C. Presl) Christenson	E			X
2. *Beloglottis costaricensis* (Rchb.f.) Schltr.	T		X	
3. *Brassavola nodosa* (L.) Lindl.	E	X		
4. *Catasetum maculatum* Kunth	E	X		
5. *Cohniella cebolleta* (Jacq.) Christenson	E			X
6. *Cyrtopodium paniculatum* (Ruiz & Pav.) Garay	T			X
7. *Dimerandra emarginata* (G.Mey.) Hoehne	E	X		
8. *Encyclia cordigera* (Kunth) Dressler	E			X
9. *Epidendrum coronatum* Ruiz & Pav.	E		X	
10. *Epidendrum stamfordianum* Bateman	E		X	
11. *Epidendrum vulgoamparoanum* Hágsater	E		X	
12. *Guarianthe skinneri* (Bateman) Dressler & W.E. Higgins	E		X	
13. *Habenaria macroceratitis* Willd.	T	X		
14. *Laelia rubescens* Lindl.	E			X
15. *Malaxis aurea* Ames	T		X	
16. *Oeceoclades maculata* (Lindl.) Lindl.	T	X		
17. *Palmorchis* sp.	T			
18. *Pleurothallis quadrifida* (Lex.) Lindl.	E	X		
19. *Scaphyglottis micrantha* (Lindl.) Ames & Correll	E	X		
20. *Scaphyglottis stellata* Lodd. ex Lindl.	E		X	
21. *Sobralia decora* Bateman	E	X		
22. *Specklinia grobyi* (Bateman ex Lindl.) F. Barros	E	X		
23. *Specklinia microphylla* (A. Rich & Galeotti) Pridgeon & M.W.Chase	E		X	
24. *Sarcoglottis* sp.	T			
25. *Trichosalpinx blaisdellii* (S. Watson) Luer	E			X
26. *Trigonidium egertonianum* Bateman ex Lindl.	E	X		

E = Epífita; T = Terrestre

meses secos. Únicamente *Palmorchis* sp. y *Oeceoclades maculata* (Lindl.) Lindl. conservan sus hojas durante todo el año. Las plantas de *Cyrtopodium paniculatum* crecen en áreas rocosas con materia orgánica en bosques secundarios. *Habenaria macroceratitis* Willd. se encuentra a orillas de caminos en zonas expuestas y alteradas o en el bosque secundario. *Sarcoglottis* sp., una especie aún no identificada, crece en grandes colonias en el interior de los bosques secundarios y zonas rocosas en sitios con sombra. Las poblaciones presentan cierta variación en la coloración de las hojas y las flores. *Beloglottis costaricensis* (Rchb.f.) Schltr., una especie poco conocida y casi nunca recolectada es fácil de observar en el PNBH usualmente en poblaciones mezcladas con *Sarcoglottis* sp.

Dentro de los hallazgos más importantes está la presencia de *Malaxis aurea* Ames, una especie poco común, conocida únicamente del bosque húmedo premontano. En el PNBH se encuentra en zonas de bosque secundario en suelos rocosos con materia orgánica. Este hallazgo representa un dato ecológico nuevo para esta especie. Una especie de *Palmorchis* todavía no identificada es también un nuevo registro en términos ecológicos para el sitio y podría tratarse de una especie no descrita (Dressler, com. pers. 2005). La especie exotica y recién naturalizada, *Oeceoclades maculata* es común en ciertas áreas del PNBH y forma

colonias a lo largo de áreas de bosque secundario. Su presencia se reporta para las áreas secas del Parque Nacional Santa Rosa y ha sido recolectada en el Pacífico Central y Sur (Dressler 2003).

Las acciones futuras del proyecto se concentran en evaluar otras áreas del PNBH pues es posible encontrar algunas especies que habitan en la misma región biogeográfica del bosque seco. Además, el material disponible de *Specklinia microphylla* (A. Rich & Galeotti) Pridgeon & M.W.Chase y *Trichosalpinx blaisdelli* (S. Watson) Luer, requiere de un estudio taxonómico detallado, siendo posible que estos nombres no estén correctamente aplicados al material encontrado. El presente estudio proporciona nueva información sobre los ecosistemas de bosque seco e intenta explicar la flora de orquídeas del PNBH basandose en la distribución general de las especies a nivel nacional. Esta información puede ser aprovechada por las demás áreas protegidas dentro el mismo ecosistema, las cuales comparten algunas especies que alcanzan una distribución más amplia. De esta manera se pretende iniciar un sistema que permita fortalecer el conocimiento y la protección de la flora de orquídeas de la región del bosque seco de Costa Rica.

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LITERATURA CITADA

Aguilar, T & P. Denyer. 2001. Una especie nueva de *Euphyllia* (Scleractinia: Caryophylliidae) en las calizas de Barra Honda (Paleógeno), Costa Rica. Rev. Biol. Trop. 49. Supl. 2: 195-201.

Atwood, J. T. 1987. The vascular flora of La Selva Biological Station, Costa Rica: Orchidaceae. Selbyana 10: 76-145.

Boza, M. A. 1986. Parques Nacionales Costa Rica National Parks. Fundación de Parques Nacionales, Costa Rica. Incafo, Madrid.

Dressler, R. 2003. Orchidaceae. Pp. 1-595 *in:* B.E. Hammel, M.H. Grayum, C. Herrera & N. Zamora (eds.). Manual de Plantas de Costa Rica. Vol. III. Orchidaceae-Zingiberaceae. Monogr. Syst. Bot. Missouri Bot. Gard. 93.

Gómez-Laurito, J. & R. Ortiz. 2004. Lista con anotaciones de las angiospermas de la Reserva Biológica Alberto Brenes (microcuencas de los ríos San Lorenzo y San Lorencito), Costa Rica. Lankesteriana 4: 113-142.

Jaccard, S., M. Münster, P.O. Baumgartner, C.Baumgartner-Mora & P. Denyer. 2001. Barra Honda (Upper Paleocene- Lower Eocene) and El Viejo (Campanian-Maastrichtian) carbonate platforms in the Tempisque area. Rev. Geol. Amér. Central 24: 9-28.

Jiménez, Q. & M.H. Grayum. 2002. Vegetación del Parque Nacional Carara, Costa Rica. Brenesia. 57-58.

Janzen, D.H. (ed.) 1983. Costa Rican natural history. Chicago University, Chicago.

Mora, S. 1978. Estudio geológico de los Cerros Barra Honda y alrededores, cantón de Nicoya, provincia de Guanacaste, Costa Rica. Tesis de Bachillerato. San José, Universidad de Costa Rica. 173 p.

Pupulin, F. 1998. Orchid florula of Parque Nacional Manuel Antonio, Quepos, Costa Rica. Rev. Biol. Trop. 46 (4): 961-10317.

Pupulin, F. 2003. Orquídeas de Tapantí. Jardín Botánico Lankester, Universidad de Costa Rica.

Tosi, J. 1969. Mapa ecológico de Costa Rica. Centro Científico Tropical, San José, Costa Rica.

Weber, A., W. Huber, A. Weissenhofer, N. Zamora & G. Zimmermann (eds.) 2001. An introductory field guide to the flowering plants of the Golfo Dulce rainforests, Costa Rica. Corcovado National Park and Piedras Blancas National Park ("Regenwald der Österreicher"). Stapfia 78: 1-464.

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POLLINATION ANALOGIES BETWEEN ORCHIDACEAE, *FICUS* (MORACEAE) AND ASCLEPIADACEAE

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RESUMEN. Las orquídeas, así como Asclepiadaceae, poseen polinios para transportar polen masivamente. Algunos de los géneros de avispas (Agaonidae), que polinizan a los higos (Moraceae: *Ficus*), poseen cavidades (sacos torácicos y corbículas coxales) donde el polen se transporta. Estos dispositivos en sendos grupos, probablemente evolucionaron para facilitar el transporte de abundantes granos de polen, por largo tiempo y distancia, sin que éste se deshidrate o se desprenda de los insectos vectores. Estas estructuras condujeron a la evolución de granos de polen pequeños, livianos, semihemisféricos, parcialmente deshidratados, sin ornamentaciones, colores y olores atractivos. La evolución de estos dispositivos condujo a la evolución de polinizadores específicos, a la especiación isopátrica, endemismo y producción de síndromes de polinización análogos. Se postula que los estigmas de *Ficus* no son receptivos y son secos; consecuentemente, los estigmas de las flores "agalla" y flores "semilla" se tornan húmedos y receptivos cuando son "picados" por el ovipositor de las avispas. Las estructuras para transportar polen en las avispas de los higos han evolucionado varias veces; y es posible que lo mismo aconteció con la evolución de polinios en las orquídeas. La evolución de estas estructuras en los organismos mencionados, puede haber sucedido durante períodos geológicos de sequía.

KEY WORDS: pollen, containers,fig,wasps, Agaonidae, Asclepiadaceae

Introduction

In the majority of animal-pollinated angiosperms, as well as insect-pollinated gymnosperms (e.g. cycads) the acquisition of pollen by the animal vector is incidental or at least not deliberate. The pollinators usually become dusted with non-coherent pollen when they contact the anthers. The loose powdery pollen is made up by monads, which are large, with well developed and heavily sculptured exine-intine. Pollen adheres to the vectors by different physical mechanisms. The pollen carrying vectors visit other flowers usually of the same plant species, and accomplish incidental pollination. The time lapse from pollen acquisition to the act of pollination usually takes a short time. Moreover, pollen is carried in the general body surface of the vectors, where it is subject to be scraped, rubbed, blown away or washed off by rain. Consequently, transport of loose pollen grains to a long distance is practically impossible. Moreover, the exposed pollen suffers dehydration (Ramírez-B. 1989; Pacini & Hesse 2002).

Dehydration of pollen during transport, as well as pollen hydration during deposition on the stigmas and germination, are critical phases. These problems are more important, especially when the vectors, as well as the stigmas are exposed to solar radiation, air currents (Pacini & Hesse 2002), or when the stigmas are dry, as probably occurs in *Ficus*. The "advanced" orchids have evolved different kinds of pollinia which act as pollen transportation containers, while some "advanced" genera of fig-wasp pollinators have evolved hidden body containers (pollen pockets and corbiculae) to transport pollen to be used in future pollination (Ramírez-B 1969; Ramírez-B & Malavasi 1997). The Asclepiadaceae have also pollinia, and are the dicot counterpart of the "advanced" Orchidaceae (Wyatt & Broyles 1994). Pacini & Hesse (2002) noted that "pollen longevity should be considered in relation to whether it is inside or outside the anther." While Jersáková *et al*. (2005) noted that "among animal-pollinated species, the fate of the transported pollen depends fundamentally on whether pollen travels as independent grains or aggregations."

Ramírez-B. (1989) postulated that the evolution of the adult shortly-lived symbiotic agaonid pollinators of *Ficus* probably saves the pollen from desiccation and death. He noted that *Ficus* species have not evolved genetic mechanisms of isolation and that isopatric speciation may have also occurred. The remarkable closed pollinating mechanisms in *Ficus* and in the orchids may have had a causal relationship to their extensive speciation and endemism (Ramírez-B. 1970a; 1986)

The objective of this work was to explain why the "advanced" orchids (as well as Asclepiadaceae) have evolved pollen containers (e.g. pollinia) and why some "advanced" pollinator genera of *Ficus* have also evolved containers to carry pollen. Compare the pollination syndrome analogies between the orchids (Orchidaceae), of *Ficus* (Moraceae), and Asclepiadaceae, as well as, to discuss their implications in the evolutionary consequences in those groups.

FICUS (MORACEAE). *Ficus* is characterized by the urceolate closed inflorescence (the syconium), and its dependence on insect pollination. It is one of the largest genera of tropical woody plants with *ca*. 750 spp. (Berg 1990). In the Urticales successful adaptation to insect pollination is only known for *Ficus*, with the pseudocarpous inflorescences (Berg 1977). Pollen or other floral rewards in *Ficus* are unknown.

The symbiotic pollinators of *Ficus* belong to Agaonidae (Hymenoptera: Chalcidoidea). At oviposition , the agaonid wasps rupture ("sting") the stigmas of all fig florets and introduce the ovipositor along the style and deposit one egg inside the fig ovary of some of them. The female wasps are usually not hairy and scarcely any pollen is found on the external surface of their bodies when they penetrate the receptive syconia (Galil & Eisikowitch 1969). Each fig species produces specific attractants when the syconia are receptive (van Noort & Compton 1996 and references therein). The inflorescences have hundreds or thousands (Condit 1920) of small uniovulate florets. Fig pollen grains are very small (Cunningham 1889) pure white (Cunningham, 1889, Condit 1920, Pemberton 1921), smooth (Verkerke 1986), spherical or slightly oval (Condit 1920).

"Primitive" *Ficus* species (e.g. *F. carica* L.) have many long pedicellate male flowers, with multiple large introrse anthers, up to six (Condit, 1920), with slender filaments (Berg 1990). They also have a well developed endothecium that allows wide opening of the anthers and explosive discharge of pollen (Galil & Neeman 1977); thus, pollen does not come out naturally from the anthers (Ramírez-B. 1970b, 1974; Galil & Meiri 1981). The enclosed new generation of wasps becomes completely dusted with pollen grains (monads) (Eisen 1896; Pemberton 1921, Ramírez-B. 1974; Galil & Meiri 1981) which are later used for pollination of the female florets of the receptive syconia of another fig tree. In the "primitive" figs, pollination is accidental and the wasps accomplish passive pollination. On the contrary, the "advanced" species of figs usually have simple stigmas, few short-pedicellate or sessile male flowers with few "small" anthers (-3) with short filaments. The endothecium is degenerate and does not actively open the anther (Galil & Eisikowitch 1968; Galil 1984); consequently, the emerging adult female wasps have to extract the pollen from the anthers by using the arolia of the fore legs and introduce it into special hidden cavities or containers, pollen pockets (Figs. 5,6,7) and corbiculae (Ramírez-B. 1969, 1970a). At oviposition, the wasps simultaneously extract pollen from the containers by means of the arolia of the fore legs. Then, the pollen grains are shaken or rubbed on the stigmas or synstigmas. In fig species with symbiotic wasps that posses pollen containers, pollination is "deliberate" and they accomplish active pollination. Galil & Eisikowitch (1971) questioned for the first time," what could have been the selective advantage that promoted the development in (the fig wasps) of unique pollen pockets and the appropriate instincts to load these pockets in old figs and empty them in receptive figs during the oviposition act?"

The female florets of *Ficus* seem to have non-receptive dry stigmas. It is known that in the "female flowers" of *Ficus. carica* L. (the edible fig), and probably other species, the stigmas are covered with multiple minute glands, which become greatly swollen and somewhat glossy of a green light color (Eisen 1896). Verkerke (1986, 1987) also noted that the stigmas of the "female" and "gall flowers" of *F. asperiifolia* Miq. and *F. ottonifolia* (Miq.) Miq. (species which have active pollination), become

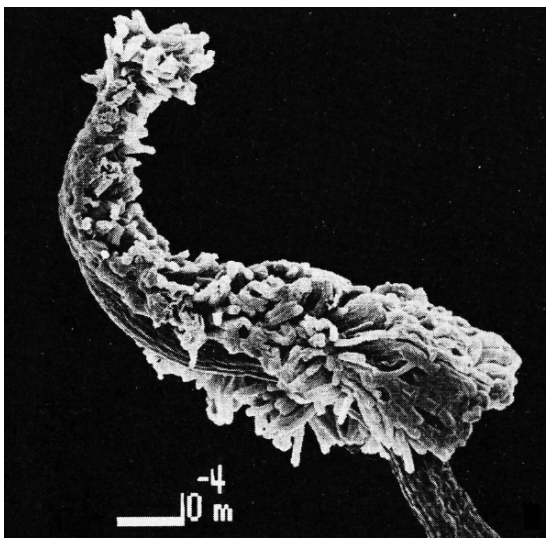

FIGURE 1. *F. ottonifolia*. Seed flower at pre-fertilization time (after Verkerke, 1986; Fig. 5d).

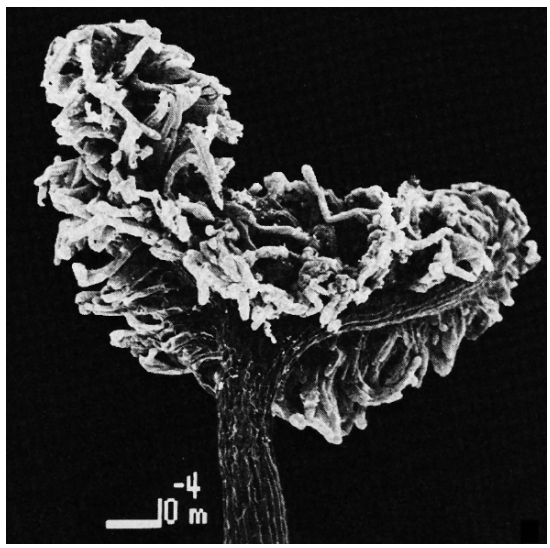

FIGURE 2. *Ficus ottonifolia* (Miq.) Miq. Gall flower at pre-fertilization time (after Verkerke, 1986; Fig. 4d).

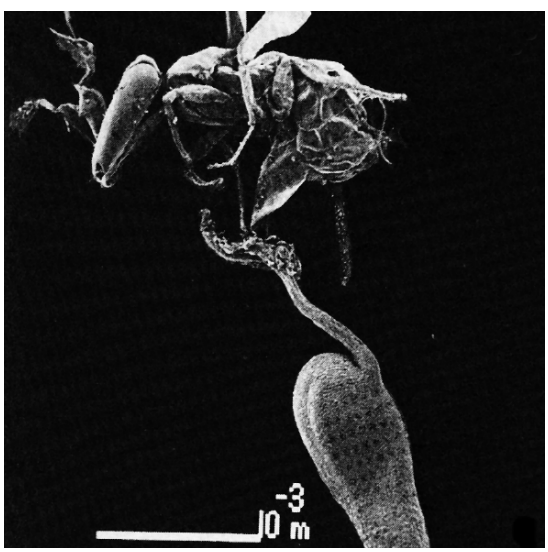

FIGURE 3. *F. ottonifolia*. Gall flower penetrated by the ovipositor of *Courtella gabonensis* Wiebes (after Verkerke, 1986; Fig. 6b).

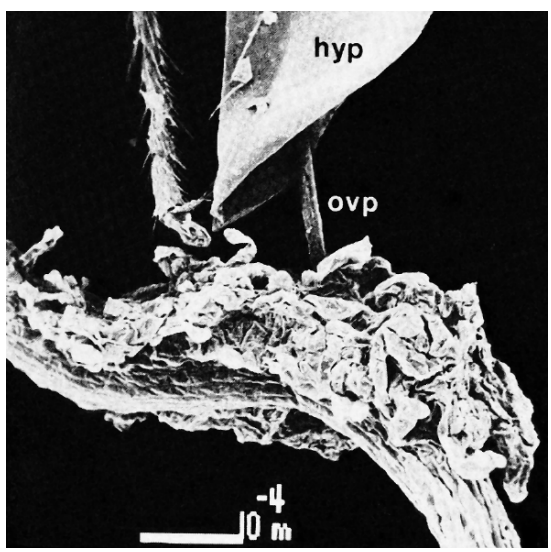

FIGURE 4. *F. ottonifolia*. Gall flower penetrated by the ovipositor of *Courtella gabonensis* (after Verkerke, 1986; Fig. 6c): hyp=hypopygium, ovp=ovipositor.

shortly papillate when receptive as in (Figs. 1, 2).

The supposed dry non-receptive stigmas of *Ficus* probably do not posses a natural sugar-rich solution or "stigmatic fluid" for pollen hydration and germination. This has been indirectly demonstrated by the successful manual pollination and the production of viable seeds when pollen-sugar solutions were introduced into the receptive syconia, of different fig species (Condit 1950; Neeman & Galil 1978; Ramírez-B. 1986). Since the fig stigmas or synstigmas are probably dry, each stigma and style have to be ruptured or "stung" by the wasps' ovipositors (Fig. 3, 4) in order to secrete a stigmatic fluid that allows pollen hydration, vacuolation, and germination; as well as pollen tube penetration and fertilization. Galil & Eisikowitch (1969) also report that the female *Ceratosolen arabicus* Mayr not only "sting" the styles but also gnaws the stigmata during oviposition.

ORCHIDACEAE. The Orchidaceae constitute one of the largest group of monocots, estimates range from 17-35 000 species (Dressler 1993). The "advanced" orchids have quite specific insect pollinators (Dressler 1968). According to Nepi *et al*. (2001) pollen reward is unknown in the orchid flowers, except in *Neuwiedia veratifolia* Bl.. Yeung (1987) noted that "the most notable feature in orchid pollen development is that the different pollen grouping can be found".

The "primitive" orchids had introrse anthers as in *Ficus*. Nevertheless, in *Cleistes divaricata* (L.) Ames, a hinged anther dispenses a sequence of loosely aggregated pollen tetrad masses… (Gregg 1991). In the "primitive" Apostasioideae, pollen is produced in loose monads (Singer *et al*., 2006). In *Neuwiedia* spp. it is powdery and not coherent (Dressler 1993). The pollen has well developed exine and intine. In *N. veratifolia Blume* (Apostasioideae) the anthers are tubular as in buzz flowers (Dressler, R, pers. comm. 2007). Pollen, as in most angiosperms is accidentally loaded and transported on the body of the pollinator agents (bees and other vectors). The stigmas of orchids with monads and tetrad pollen, is more or less humid as that of other angiosperms (Pacini & Hesse, 2002). In Apostasioideae pollination must be accidental.

In the more "advanced" orchids pollen grains are agglutinated in different ways, e.g. in *Phragmipedium* (Cypripedoideae) are monads, sticky and pastelike or united into pollinia (Dressler 1993); in other Orchidaceae pollen is agglutinated in soft or divisible pollinia and in the Epidendroideae indivisible pollinia (Singer *et al*. 2006). Pacini & Hesse (2002) noted that "(orchid) pollen longevity should be considered in relation to whether pollen is exposed or protected", and that "different kinds of pollinia are found exclusively in Orchidaceae" (Pacini & Hess 2002 and reference therein).

Massulate orchids have little or no locular fluid and pollen hydration, unlike other angiosperms, and rehydration occurs inside the closed cavity of the stigma (Pacini & Hess 2002 and reference therein). The smaller size in orchid pollen is due to the lack of the vacuolated stage that is so common in angiosperm pollen (Pacini & Hesse 2002). They also noted that "if pollen increased as much as much of that of other angiosperms, it would be impossible to have a compact pollinium."

ASCLEPIADACEAE. The Asclepiadaceae are the dicot counterpart of the Orchidaceae, which also transmit the pollen in large groups within pollinia (Wyatt & Broyles 1994). They produce capsules with hundreds of seeds. Milkweed flowers are long-lived and produce copious nectar which flows from nectaries within the stigmatic chamber. Nectar also serves as the germination fluid for pollen grains (Wyatt & Broyles 1994). Most milkweeds species are genetically incompatible (Wyatt & Broyles 1994), and they seem to depend on quite specific pollinators.

Discussion

In most of the animal-pollinated angiosperms, as well as those insect pollinated gymnosperms (e.g. cycads), the mature anthers, split open longitudinally to release the pollen (Yeung 1987) and the acquisition of pollen by the animal vectors is accidental. The loose ornamented and reticulated pollen grains have well developed exine and intine and become adhered to the vector by different physical mechanisms. The pollen-carrying vector visit other conspecific flowers, and accidentally rubs the pollen in the stigmas; thus, pollen usually remains on the vector for quite short time. Since pollen is carried on the general vectors' body surfaces, it is subject to be scraped, rubbed, blown; or washed off, as well as to dehydration and death; consequently, the transport of pollen in heavy masses or simple units (monads) for a long time and distance is practically impossible.

Pollen dehydration during the transport, as well as, pollen hydration, pollen tube emergence and penetration through the stigmatic styles are critical phases. This is especially important when the stigmas are exposed to solar radiation and air currents or when they do not possess stigmatic fluid. In the genus *Ficus* the pollinators of the more "primitive" genera, most of the powdery pollen is transported as monads on the general surface of the pollinator, or between the intersegmental folds (Galil & Neeman 1977).

However, the pollinators of several "advanced" Old World fig genera have evolved discrete pollen containers, pockets (Figs. 5-7) and corbiculae (Ramírez-B. 1969, 1997). These structures are deliberately loaded at pollen acquisition and unloaded during pollination. In those containers the fig-wasps may

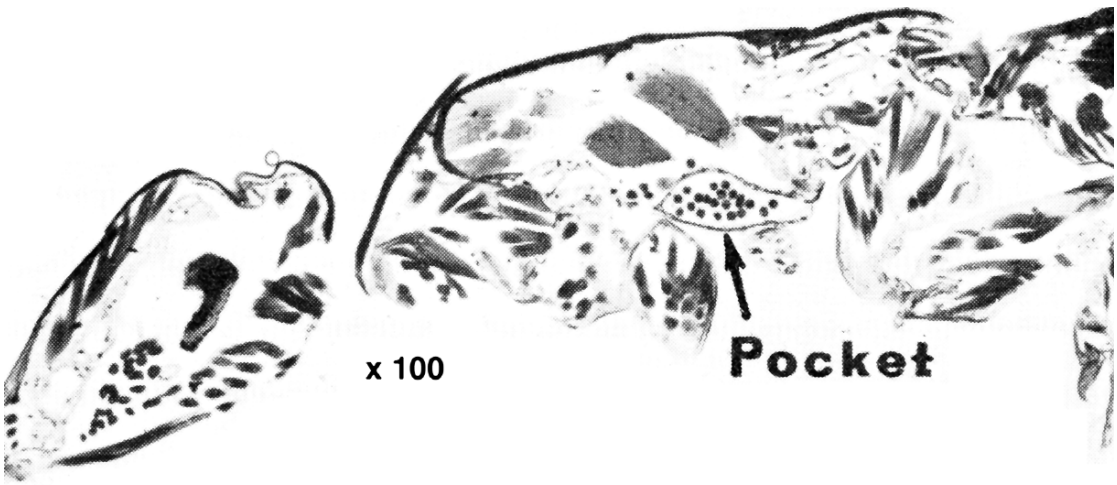

FIGURE 5. *Blastophaga quadraticeps* L., the pollinator of *Ficus religiosa* L. Longitudinal section of female showing pollen pockets (after Galil and Eisikowitch, 1970; Fig. 9).

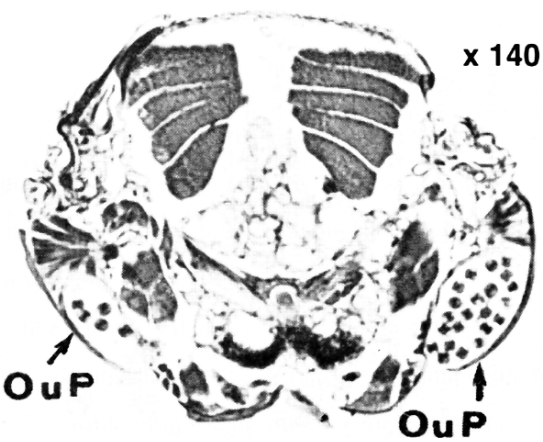

FIGURE 6. *B. quadraticeps*. Transverse section of mesothorax showing pollen pockets (after Galil and Eisikowitch, 1970; Fig. 11): Oup=outer plate.

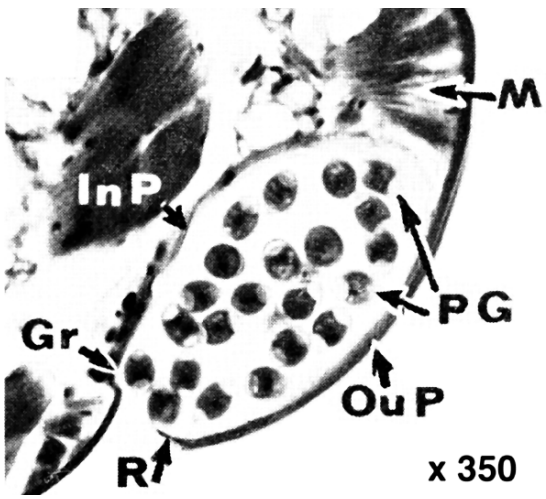

FIGURE 7. *B. quadraticeps*. Transverse section of pollen pocket (after Galil and Eisikowitch, 1970; Fig. 10): M=muscle, PG= pollen grains, Oup = outer plate, R = rim, Gr = groove, InP = inner plate.

carry hundreds or thousands of pollen grains (Fig. 7). Ramírez-B. (1989) postulated that the evolution of short-lived fig pollinator, as well as of discrete pollen transporting containers in some of the fig wasps was a mechanism to protect the pollen grains from desiccation, since there is a lapse of hours or days between pollen acquisition and pollination. This lapse of time between pollen acquisition and pollination is also a common fact in "advanced" orchids. In the "primitive" orchid (e.g. Apostasioideae) the loose pollen, as in the "primitive" figs, is probably transported on the general body of the pollinating insects (probably bees).

The "advanced" orchids, as well as the species of *Ficus* are associated with very specific insect pollinators that isolate and prevent hybridization between compatible populations. This close association has led to fast speciation without geographical isolation (Ramírez-B. 1970a) and to endemism. Since fig species as well as the "advanced" orchid (and probably Asclepiadaceae) species are pollinated by quite constant pollinators they do not seem to have evolved genetic

TABLE 1: Common analogies in Orchidaceae and *Ficus* (Moraceae).

Disruption in the production of pollen monads.
Flower anthesis last from few hours to several weeks (Endress 1994).
Elimination of bright attractive pollen colors.
Reduction of exine, intine layers and pollen ornamentation.
Elimination of pollen reticulations.
Production of small uniform semispherical light pollen grains.
Partial or total elimination of locular fluid.
Production of partially or totally dehydrated pollen grains, or lack of the vacuolated stage.
Reduction of the number of male flowers and anthers per male flower.
Synchronous development, production and maturation of pollen, and extension of flower anthesis (Endress 1994).
Prevention of pollen dehydration and death (Ramírez-B. 1989).
Vacuolization of pollen on the stigmas (Pacini & Hess 2002).
The pollinators carry more pollen grains per load and for longer periods.
Elimination of mechanical or liquid attachments (Pacini & Hesse 2004).
Prevention of pollen grains from being blown away, scrapped, rubbed or washed off during transport to the stigma.
Prevention of pollen waste.
Pollen may survive for longer times before pollination and pollen germination (Nepi *et al*. 2001)
Production of long-lived flowers, especially in orchids.
Evolution of very specific pollinator adapted to the flowers morphology in orchids or to the inflorescences physiology and morphology in *Ficus*.
Evolution of sympatric speciation and endemisms.
Reduction or elimination of anthers' locular fluids.
Reduction or fusion of floral parts.
The evolution of specific floral attractants and morphologies that fitted the behavior, morphology and size of the pollinator.
Evolution of bizarre and colorful flowers in the orchids and of intricate ostiolar entrances in *Ficus*.
Increased number of ovules per capsule (Pacini & Hesse, 2002) in the orchids; as well as the number of female flowers per syconium of *Ficus* species.
Reduction of geitonogamous pollination (Johnson & Edward 2000).
Production of stigmatic complexes in the orchids and Asclepiadaceae and of synstigmas in *Ficus*.
Increase of cross pollination (Jersáková *et al*. 2006).
Synchronous fertilization of hundreds, thousands or millions of orchid ovules, or of hundreds or thousands of female fig florets in a single pollination act.
Inhibition of promiscuous pollination.
Production of seeds from a single father (Wyatt & Broyles 1994)
Production of very small seeds.
Evolution of symbiotic germination and epiphytism.

mechanisms of isolation. Instead of, *Ficus* and the "advanced" orchids evolved physiological, mechanical, ecological, an ethological mechanisms of isolation.

The evolution of discrete pollen-carrying containers (pollinia) in the orchids and in some of symbiotic pollinators of *Ficus* (pollen pockets and corbiculae)

has had an evolutionary impact in the phylogeny of both groups; as well as in their insect pollinators. It led to the production of multiovulate orchid ovary and to fig inflorescences with hundreds or thousands of ovules or female florets and few male flowers. The fig syconium is analogous to the multiovulate orchid and *Asclepias* ovaries, and the ripe inflorescence to the orchid or *Asclepias* capsules.

It is probable that the orchid pollinia may have evolved several times, while in the pollinators of *Ficus*, they seem to have evolved independent in at least in six lines of wasps, as a case of convergence (Ramírez-B. 1978). However, according to Machado *et al*. 2001, those structures evolved only once and had been lost in several agaonid wasps due to reversals. The evolution of pollen transporting devices, both in plants and animals, may have occurred during geological climatic dry spells.

The evolution of pollen containers in some of the orchids and in some symbiotic fig wasps have also contributed to he appearance of analogous syndromes (Table 1), which probably also occurs in the Asclepiadaceace. The evolutionary pattern in the "advanced" orchids, as well as in *Ficus*, has probably being influenced by their unique pollinating mechanisms. Since the female florets of *Ficus* seem to have dry stigmas, it is postulated that the stigmas must be ruptured ("stung") by the pollinating wasp's ovipositor, or that biting of the stigmas induce the production of the stigmatic liquid that allows pollen germination.

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LITERATURE CITED

Berg, C.C. 1950. An interspecific hybrid in Ficus. J. Hered. 41: 165-68.

Cunningham, D.D. 1889. On the phenomena of fertilization in *Ficus roxburghii*, Wall. Ann. Roy. Bot. Gard. Calcutta 1: 13-51.

Berg, C.C. 1977. Urticales, their differentiation and systematic position. *Plant Sys. Evol.* Suppl. 1: 349-74.

Berg, C.C. 1984. Floral differentiation and dioecism in *Ficus* (Moraceae). *Mini Symposium Figs and Fig Insects*, p. 15-25. Montpellier Cédex: Centre National de la Recherche Scientifique.

Berg, C.C. 1990. Reproduction and evolution in *Ficus* (Moraceae): traits connected with the adequate rearing of pollinators. Mem. New York Bot. Gard. 55: 169-85.

Condit, I.J. 1920. Caprifigs and caprification. Univ. Calif. Exp. Sta. Bull. 319: 341-75.

Dressler, R. L. 1968. Pollination by euglossine bees. Evol. 22(1): 202-10.

Dressler, R. L. 1993. Phylogeny and classification of the orchid family. 314 pp. Portland: Dioscorides Press.

Eisen, G. 1896. Biological studies on figs, caprifigs and caprification. Calif. Acad. Sci. (Second Series) 5: 897-1003.

Endress, P.K. 1994. Diversity and evolutionary biology of tropical flowers. 500 p. Cambridge: Cambridge University Press.

Galil, J. 1973. Pollination in dioecious figs: pollination of *Ficus fistulosa* by *Ceratosolen hewitti*. Gardens' Bulletin 26(2): 303-11.

Galil, J. 1984. Anther dehiscence in fig syconia. Mini Symposium Figs and Fig Insects, p. 35-40. Montpellier Cédex: Centre National de la Recherche Scientifique.

Galil, J. & D. Eisikowitch. 1969. Further studies on the pollination ecology of *Ficus sycomorus* L. Tijdschr. Entomol. 112: 1-13.

Galil,Y. & Snitz-Pasternak. 1970. Pollination in *Ficus religiosa* L. As connected with the structure and mode of action of the pollen pockets of *Blastophaga quadraticeps* Mayr. New Phytol. 69: 775-784.

Galil, J. & D. Eisikowitch. 1971. Studies on mutualistic symbiosis between syconia and sycophilous wasps in monoecious figs. New Phytol. 70: 773-87.

Galil, J. & L. Meiri. 1981. Number and structure of anthers in fig syconia in relation to behaviour of the pollen vectors. New Phytol. 88: 83-87.

Galil, J. & G. Neeman. 1977. Pollen transfer and pollination in the common fig (*Ficus carica* L.). New Phytol. 79: 163-171.

Greeg, K.R. 1991. Reproductive strategy of *Cleistes divaricata* (Orchidaceae). Am. J. Bot. 78: 350-360.

Jersáková, J., S.D. Johnson & P. Kindlmann. 2006. Mechanisms and evolution of deceptive pollination in orchids. Biol. Rev. 81: 1-17.

Johnson, S.D. & T.J. Edwards. 2000. The structure and function of orchid pollinaria. Plant Syst. Evol. 203: 91-98.

Machado, C.A., M. Jousselin, F. Khellberg, S.G. Compton & E.A. Herre. 2001. Phylogenetic relationship, historical biogeography and character evolution of fig–pollinating wasps. Proc. Roy. Soc. London – Biol. Sci. 268: 685-694.

Neeman, G. & J. Galil. 1978. Seed set in the 'male syco-

nia' of the common fig *Ficus carica* L. (Caprificus). New Phytol. 81: 375-80.

Nepi, M. G.G. Franchi & E.P. 2001. Pollen hydration status and dispersal: cytophysiological features and strategies. Protoplasma 216: 171-180

Noort van, S. & S.G. Compton. 1996. Convergent evolution of Agaonine and sycoecine (Agaonidae, Chalcidoidea) head shape in response to the constraints of host fig morphology. J. Biogeogr. 23: 415-24.

Pacini, E. & M. Hesse. 2002. Types of pollen dispersal units in orchids, and their consequences for germination and fertilization. Ann. Bot. 89: 653-64.

Pemberton, C.E. 1921. The fig wasp in its relation to the development of fertile seed in the Moreton Bay fig. Hawaiian Plant. Rec. 24: 297-319.

Pridgeon, A. M., P.J. Cribb, W.W. Chase & F.N. Rasmussen. 1999. Apostasioideae. Pp. 94-96 . *in*: A. M. Pridgeon, P. J. Cribb, W. W. Chase & F. N. Rasmussen (eds.). Genera Orchidacearum. Vol. 1. Oxford, Oxford University Press.

Ramírez-B., W. 1969. Fig wasps: mechanism of pollen transfer. Science 163: 580-81.

Ramírez-B., W. 1970a. Host specificity of fig wasps (Agaonidae). Evolution 24(4): 680-691.

Ramírez-B., W. 1970b. Taxonomic and biological studies of neotropical fig wasps. Univ. Kans. Sci. Bull. 49, no. 1: 1-44.

Ramírez-B., W. 1974. Coevolution of *Ficus* and Agaonidae. Ann. Missouri Bot. Gard. 61: 770-780.

Ramírez-B., W. 1978. Evolution of mechanisms to carry pollen in Agaonidae (Hymenoptera: Chalcidoidea). Tijdschr. Entomol. 121(6): 279-93.

Ramírez-B., W. 1986. Artificial hybridization and self-fertilization in *Ficus* (Moraceae). Brenesia 25/26: 265-72.

Ramírez-B., W. 1989. Dispersal and colonization of *Ficus* in the New World. Pp. 279-84 *in*: P.R. Crane & S. Blackmore (eds.). Evolution, Systematics, and Fossil History of the Hamamelidae, vol. 2: 'Higher' Hamamelidae. Oxford: Clarendon Press.

Ramírez-B., W. & J. Malavasi. 1997. Fig wasps: mechanisms of pollen transfer in *Malvanthera* and *Pharmacosycea* figs (Moraceae). *Rev. Biol. Trop.* 45(4): 1635-40.

Singer, R.B., A.J. Marsaioli, A. Flach & M.G. Reis. 2006. The ecology and chemistry of pollination in Brazilian orchids: recent advances. Vol. 1. Pp. 569-82 *in*: J.A. Teixeira da Silva (ed.). Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues. Londron, Global Science Books.

Verkerke, W. 1986. Anatomy of *Ficus ottoniifolia* (Moraceae) syconia and its role in the fig-fig wasp symbiosis. Proc. K. Ned. Akad. Wetensc. 89(4): 443-69.

Verkerke, W. 1987. Syconial anatomy of *Ficus asperiifolia* (Moraceae) a gynodioceious tropical fig. *Proc. K. Ned. Akad. Wetensc.* 90(4): 461-92.

Wyatt, R., & S.B. Broyles. 1994. Ecology and evolution of reproduction in milkweeds. Annu. Rev. Ecol. Sys. 25: 423-41.

Yeung, E.C. 1987. Development of pollen and accessory structures in orchids. Pp. 193-226 *in*: J. Arditti (ed.). Orchid Biology: Reviews and Perspectives. Vol. IV. Ithaca, New York, Cornell University Press.

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ONCIDIUM SURPRISES WITH DEOXYRIBONUCLEIC ACID

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There is an armchair taxonomist I know that wrote that the taxonomy of the Oncidium alliance was a *mess*. I'd been thinking that the remark was based on lack of knowledge. In my opinion, he is way off base. Know that I am *not* a taxonomist… only a grower and an artist. But I do believe that the Oncidium alliance is a reasonably well organized taxonomy.

My first orchid was purchased from Jones and Scully in 1962. Around 1980 I began growing species in the Oncidium alliance and illustrating the plants and flowers. I have painted actual size portraits of more than 850 plants and flowers in *Oncidium* as well as other genera. When one paints an orchid flower, it is visually dissected for accurate illustration. This leads to a modicum of knowledge, but I still consider myself an amateur. But what I do know is that botanical taxonomy has taken a turn for the better and has become a scientific, rather than an intuitive, process for classification.

For many years, Norris Williams and Mark Chase have been working with deoxyribonucleic acid or DNA which is the key molecule responsible for defining heredity in plants (as well as in humans) for orchids in the Oncidium alliance; Norris in Gainesville, Florida and Mark at the Jodrell Laboratory at Kew of which he is the head. What they have been finding with molecular sequencing has made for a number of surprises. Their molecular analysis has even moved groups of species from one genus to another.

As an example, the Brazilian monotype we have known for years as *Oncidium phymatochilum* has been moved into *Miltonia* based on their studies. The species is now *Miltonia phymatochila*. This orchid can produce inflorescences with hundreds of gossamer flowers.

Mexicoa gheisbrechtianum, now *Oncidium gheisbrechtianum*, has now been returned to *Oncidium* (where it was originally placed) and is a member of a group of Oncidium species from Mexico and Central America.

Another Mexican and Central American species, *Amporoa beloglossum*, is now in genus *Rhyncostele.*

Another monotype, *Oncidium onustum*, was studied by Williams and Chase and with DNA research and other observations, they confirmed that because there were a number of differences with this species, it was removed from the body of *Oncidium* and it is now called *Zelenkoa onusta*. They grow on cacti as well as trees in southern Ecuador and northern Peru.

All of these taxonomic relocations were surprises, and when I got over the shock, they were included in the first and second edition of *The Pictorial Encyclopedia of Oncidium.* The book was edited by Mark Chase. The book cogently presents the major groups in the Oncidium alliance and has been proven useful as a reference book. More surprises have been included with many in the Oncidium alliance moved into new groups, their relationships defined and verified with DNA and included in the book with a DNA tree. With the changes made by Williams and Chase, *Oncidium* is far from being a "mess" as the book demonstrates.

Here's another surprise: *Cyrtochilum,* until recently, was generally accepted to cover a group of large-flowered *Oncidium*. But based on DNA analysis, some groups that were formerly in *Oncidium* and *Odontoglossum* have been moved into *Cyrtochilum* which now includes the species in the groups called *Myanthum, Cimiciferum*, *Ramosissimum*, *Angustatum, Aureum,* and *Buesiella*, along with the large-flowered *Cyrtochlium* which, for convenience, we call the Macranthum group. Though separate from O*ncidium*) they all, of course, remain in the Oncidium alliance.

The large-flowered *Cyrtochilum*, first described by Kunth in 1815, was based on the discovery of a plant by Humboldt and Bonpland in Colombia during their Andes exploration around the beginning of the nineteenth century. The plant became the type for a group of about fifty species that are found mostly in the Andes of Venezuela, Colombia, Ecuador, Bolivia and Peru with one species found in the Caribbean.

In 1990 I met Alex Hirtz in the States and he invited me to the Guayaquil orchid show the following year. I went and I saw a magnificent plant labeled *Odontoglossum eduardii.* To me, the flowers did not remotely resemble an *Odontoglossum*. Because the flowers of *O. eduardii* had triangular or shield-shaped lips, they more closely resembled flowers in the Cimiciferum group. Around that time, Carl Withner wrote about the species in the American Orchid Society magazine and they were then still called *Odontoglossum*. I illustrated two forms of the species and Mark agreed they belonged in *Cimiciferum*, and so were placed there in the original edition of our book published in 1997. DNA research has since confirmed that this species does belong there. This was a minor surprise… however there were other surprises that I had a hard time accepting when I learned of them. Two groups that, at first, did not seem to me to be anywhere near each other in the *Oncidium* lexicon were *Otoglossum* and *Globuliferum*. Some traditional taxonomists have questioned the findings because, on the surface, they have physical features that seem dissimilar. But I later noted that these orchids also have similar features that bring them closer together. With DNA research, Williams and Chase found that the groups are so closely related genetically that they should be combined into a single genus, as they were.

Another surprise I had a difficult time with was the *Erycina* genus. The genus now includes *Erycina cristagalli* along with *Erycina pusilla* and *Erycina glossomystax* (formerly both known as *Psygmorchis* and earlier *Oncidium*) in addition to *Erycina echinata*, and *Erycina hyalinobulbon*. All are twig epiphytes. *Erycina cristagali* and E*rycina pusilla* and *Erycina glossomystax* are very close in many respects. *Erycina cristagalli* is from Central America while the others are very widespread and occur from Central America to Venezuela, the Guayanas and extend west and south in South America as far as Bolivia. These species usually grow in dappled light with high humidity and are rather soft-leaved. But *Erycina echinata* from Mexico grows quite a bit dryer in bright light and its leaf texture feels harder than the former species. Although the floral characteristics for *Erycina cristagalli* and *Erycina psygmorchis* are very similar… it is hard to tell the flowers apart… they seemed rather far apart from *Erycina echinata*, but I was reassured by Norris that their DNA work verified the relationship without question.

Taxonomic tradition, based on intuition combined with knowledge, had usually placed orchids with obviously similar characteristics into groups. The "mule ear" and "rat-tail" *oncidium* were always treated as separate entities. Their physical differences seemed obvious and this kept them apart. However, molecular analysis has changed this and folded the two groups into genus *Trichocentrum*.

Another surprise includes a small group of large, widespread, warm-growing plants that can produce large quantities of flowers. Species in this group range from Central America south to Peru and were previously classified as O*ncidium*. They are now a new genus based on their DNA; *Cyrtochiloides*, in which there are possibly four species… C*yrtochiloides cardiochila* and *Cyrtochiloides ochmatochila* might be geographic varieties of a single species. There's also *Cyrtochiloides riopalenquense* and *Cyrtochiloides panduriforme*. The Ecuadorian species, *Cyrtochiloides riopalenquense* produces a branched inflorescence that can exceed twelve feet (about four meters) with as many as a hundred flowers.

Williams and Chase also have found that the species in *Helcia*, *Leucohyle* and *Neoescobaria* are members of *Trichopilia*. Other authors had treated them as species of *Trichopilia* in the past, so it was not too great a surprise. Their work, however, confirms the definitive relationship of these plants without question in my mind.

Here's another surprise that's easy to live with: The species of *Cochlioda* and *Symphyglossum* are considered *Oncidium* by Mark and will be so listed in the future rather than as separate genera.

Now we come to what had been known for years as *Oncidium cucullatum*. A small-flowered brown and white orchid first found near Cauca, Colombia and named *Caucaea radiata* was described years before *Oncidium cucullatum*. Williams and Chase found that with molecular analysis *Caucaea radiata* fell right in the center of the species of *Caucaea olivaceum* they tested (which is the correct name for *Oncidium cucullatum*). It was another surprise to me because the flowers seemed so different. The well-known taxonomist, John Stacy, listed twenty-two species and varieties related to *Oncidium cucullatum* in his monograph that was echoed by another renowned taxono-

mist who had not studied them in the field but produced a retread of Stacy's work in the America Orchid Society magazine. When Stacy visited Ecuador more than thirty years ago, he could not have seen many populations because fewer roads were open then as compared with today. I disagree with Stacy's designation of the number of species and believe that there are far fewer than he claims. Stacy gave species status to a plant with a branched inflorescence, another to one with a straight raceme, and another to one with a zigzag spike, although the flowers of all three are similar. Among the more than fifty *Caucaea* plants from all over Ecuador and Colombia that I grow, I had one with *all three* forms of inflorescence on a single plant. Either he was not correct or I have a *Caucaea* with all three species. Some of the flowers in *Caucaea* have different lip calli, but according to Mark Chase, this may not define a species. In the *Caucaea* genus, including *olivaceum*, I believe that there are only *Caucaea sanguinolentum*, *Caucaea mimetica*, *Caucaea nubigena* and *Caucaea phalaenopsis* as good species and most of Stacy's "species" names are for geographic varieties. Okay… some of the plants in the group *might* be different species. We hope to learn more about them one day with DNA analysis.

Stig Dalstrom has researched and written about *Odontoglossum* and offered the opinion, based on extensive studies, that many of the so-called species are, in reality, geographic varieties. I agree with him. *Odontoglossum harryanum* ranges from Colombia to Peru. It was separated from *Odontoglossum wyattianum*, in part based on the two having different column wings. Stig discovered a *harryanum* in southern Ecuador with the same column wings as *wyattianum*. *Wyattianum* is therefore the southern form of *harryanum* and is treated in our book as a variety.

I have a hard time thinking of *Odontoglossum* as being separate from *Oncidium*. Having grown both genera for more than twenty five years, to me, the differences that have divided them are so minor that I think it possible they will one day be combined. I know that Mark & Norris also believe this. Years ago, Beer put them all together into *Oncidium*, so it would be easy to consider them all back there again. DNA findings and not a propensity for "lumping" would be the basis for doing so in my opinion.

There are taxonomists who have produced excellent work over the years that do *not* agree with the findings of molecular analysis. They question the accuracy of DNA readings. But new science is hard to refute. For me, there's no room for compromise. I have bitten the DNA bullet and have come to believe that this form of classification is here to stay, that is, until something better comes along. But I'll be surprised if that happens.

Harry Zelenko was born in 1928 in New York City. He was trained as an artist at the High School of Music & Art followed by the Art Student's League and courses in art at New York University. At the age of nineteen he became art director of the largest advertising agency in Connecticut. Zelenko Associates, a creative a design group, was founded in 1953. His work has received many professional awards and he has lectured and exhibited internationally. In New York, he built orchid greenhouses on the roof and terrace of his brownstone where he grew about three thousand orchids. In 1984 he began producing paintings for a book on orchids, and as Z A I Publications, published the first edition of *The Pictorial Encyclopedia of Oncidium* in 1997 with more than 850 paintings of plants and flowers. The second, revised edition followed seven years later. He moved to Ecuador and built two greenhouses to house a tripled collection of orchids. He is currently a member of the Greater New York Orchid Society and the Quito Orchid Society.