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**PROCEEDINGS OF
THE THIRD SCIENTIFIC CONFERENCE
ON ANDEAN ORCHIDS**

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The editors

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PREFACE

After the tremendous successes of the First Scientific Conference on Andean Orchids in Gualaceo and the Second Conference at Universidad Técnica Particular de Loja, the organizers of the series received a bid from the Orchid Society of Quito, Botanical Garden of Quito, and Municipality of Quito to host the Third Conference and Show in February 2009 at the Itchimbia Cultural Center. Soon thereafter we received promises of additional sponsorship from the Ecuadorian Ministries of the Environment and Ministry of Tourism, the Botanical Foundation of the Andes, and the Catholic University of Quito. All these generous sponsors made it possible for us to invite 32 speakers from Ecuador, Brazil, Costa Rica, Mexico, the United States and Puerto Rico, Canada, United Kingdom, Germany, and Czech Republic. Lectures and poster sessions in orchid systematics, ecology, and conservation science spanned three days, February 5-7, 2009.

It is appropriate that we held the Conference in Ecuador in 2009, the bicentenary of the birth of Charles Darwin (February 9, 2009) and the 150th anniversary of publication of his *On the Origin of Species by Means of Natural Selection, or the Preservation of the Races in the Struggle for Life* (November 22, 1859). For it was largely because of his collections and observations of finches and mockingbirds in the Galapagos Islands that he became aware of morphological differences among them, differences that translated into recognition of different species on each of the islands in the archipelago and would later be adduced to support his theory of natural selection. The polymathic contributions of Charles Darwin are celebrated in this volume, some explicitly as in the papers by Ken Cameron (orchid pollination) and Stephen Kirby (geology and its relationship to orchid diversity in the Neotropics) but many others implicitly, whether they are papers devoted to systematics or ecology.

The variation in nature that serves as the raw materials for descent with modification is under assault around the world by rampant deforestation — especially in Ecuador and Brazil. Alex Hirtz addresses the problem in Ecuador, while Thomas Miranda and Philip Seaton discuss *in situ* and *ex situ* solutions, whether already in progress or proposed. As always, education is the underlying key to all the solutions, and it is in that context we hope this volume (and others in the series) will be consulted by present-day students and serve a purpose in the years ahead.

ALEC M. PRIDGEON

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ALEC M. PRIDGEON

HUGO GUILLERMO NAVARRETE ZAMBRANO

DARWIN'S ORCHIDS: THEIR VARIATION, PLASTICITY, AND NATURAL SELECTION

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ABSTRACT. Patterns of variation and their fitness consequences are critical in revealing natural selection. One of the most variable groups of plants are the deception-pollinated orchids, pollinators of which are deceived in their search for a food reward. Negative frequency-dependent selection and disruptive selection have been suggested as the means by which high levels of variation are maintained, yet in most cases such selection has not been detected, prompting alternative explanations including genetic drift. Could phenotypic plasticity cloud the effects of selection? Using a *Tolunnia variegata* population as a model system, we conducted a reciprocal transplant experiment to determine the effects of light environment on vegetative, inflorescence, and floral characteristics over multiple seasons. The results were complex and showed significant responses to light for most traits measured, but often those changes were obscured by variable responses across years, likely a consequence of dramatically different rainfall. Fruit production was similar for sun and shade sites, but trajectories of selection on a given trait between the two sites were often incongruent. Our data indicated that selection in a heterogeneous environment can indeed be blunted by phenotypic plasticity, but not all characters respond in the same way.

RESUMEN. Las consecuencias de los patrones de variación en el éxito de una planta son críticas para revelar la selección natural. Uno de los grupos más variados de plantas son las orquídeas que llevan a cabo polinización por engaño las mismas engañan a los polinizadores los cuales buscan una recompensa alimentaria en ellas. La selección negativa dependiente de frecuencia y la selección disruptiva han sido sugeridas para explicar el mantenimiento de altos niveles de variación. En la mayoría de los casos estas selecciones no han sido detectadas, provocando otras explicaciones alternas como la deriva genética. ¿Podrá la selección natural ser nublada por la plasticidad fenotípica? Usando poblaciones de *Tolunnia variegata* como sistema modelo, nosotros realizamos un experimento de trasplante recíproco para determinar los efectos de la luz en las características de la parte vegetativa, inflorescencia y flores, a través de diferentes épocas del año. Los resultados fueron complejos, la mayoría de los rasgos medidos presentaron respuestas significativas a la luz, pero la mayoría de estos cambios fueron oscurecidos por respuestas variadas a través de los años, probablemente por una diferencia dramática en la precipitación. La producción de frutos fue similar entre sitios de sombra y sol, pero las trayectorias de selección de las características entre los dos lugares fueron en su mayoría incongruentes. Nuestros datos indican que la selección en un ambiente heterogéneo puede ser disfrazada por la plasticidad fenotípica, pero no todas las características responden de la misma manera.

KEY WORDS: Orchidaceae, Charles Darwin, floral variation, deceit-pollination, natural selection

One hundred and fifty years ago, Darwin published his treatise, *On the Origin of Species by Means of Natural Selection*. Unquestionably, this was a landmark event that shook the tenets of science and

western society. This 450-page abstract established the foundation for evolutionary theory by describing the process by which change may occur through natural selection (Darwin, 1859). As the story has been told so

many times from a plethora of perspectives, Darwin was slow to amass supporting data and was pushed to come forth with his thesis when Alfred Russel Wallace wrote to him expressing essentially the same ideas (Wallace, 1858). Although Darwin did not reach the biological diverse regions of the tropical Andes, he was strongly influenced by his visit to another part of Ecuador: the Galapagos Islands (Darwin, 1845). From those studies and many others throughout the world he developed a sense of natural history perhaps unmatched by all those who preceded him and those who followed.

Over time, we have come to know Darwin's theory of natural selection quite well and have been able to enrich it with accumulated knowledge of biology, especially with a better understanding of genetics. Natural selection is now viewed as a process with three conditions. First, a population must have variation, a cornerstone to the theory thoroughly appreciated by Darwin, though he did not know the genetic basis for it. Secondly, variants within a population must show differential reproductive success, also known as fitness. And finally, the traits in question must be heritable. All three ideas were well developed by Darwin, which is remarkable considering modern genetics had not yet arrived. If all three conditions are met, then natural selection ensues with a predictable outcome (Endler, 1986).

Darwin did note that much variation came from cross-fertilization and that many plant traits were adaptations that ensured or enhanced the probability for cross-pollination. The amount of data he accumulated on this was prodigious, and his favorite model system was unquestionably orchids. He left the orchids out of the *Origin of Species*, but the first book published after his landmark treatise was *On the Various Contrivances by which British and Foreign Orchids Are Fertilised by Insects*, a compendium of pollination mechanisms associated with ensuring cross-fertilization (Darwin, 1862). He regarded those mechanisms, some quite fantastic, as a consequence of the advantages for cross-pollination. Such observations are convincing, although they merely represent indirect evidence for natural selection.

The mechanisms for cross-fertilization abound in plants, but Darwin knew that it was insufficient simply to catalogue them, that he also had to show what the

advantage was. Out came yet another book in support of his theory of natural selection: *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom* (Darwin, 1876). With meticulous garden experiments on a variety of plants, Darwin demonstrated that cross-fertilization produced strong, variable offspring, whereas repeated self-fertilization produced progressively fewer and weaker progeny, a trend that could be reversed with a return to cross-fertilization. We interpret the results of selfing as simply inbreeding depression, though Darwin expressed it more elegantly in the flowery language of the time: "It is hardly an exaggeration to say that Nature tells us, in the most emphatic manner, that she abhors perpetual self-fertilisation" (Darwin, 1862).

Orchids and floral variation

Orchids are not only an ideal model system to elucidate the remarkable adaptations associated with cross-pollination, but they are also ideal because they are perhaps the most species-rich family of flowering plants. This diversity has often been attributed to adaptive radiation associated with diverse pollination biology. Although the family is relatively old (Ramírez *et al.*, 2007), we perceive it as a dynamic group where natural selection should be pervasive.

Unlike Ponce de León, some evidence indicates that orchids have found the fountain of youth where diversification remains an active process: their populations often show considerable variation, one of the basic conditions for natural selection. Flowers may vary in size, shape, color, and even fragrance (Ackerman, 1986).

Why do many species of orchids show high levels of variation? There are several possibilities. First, there could be a breakdown in reproductive isolation mechanisms among closely related species. For example, environmental changes could lead to pollinator-sharing, creating hybrid swarms through introgressive hybridization. Certainly human alterations to the environment can create intermediate habitats that bring two otherwise separated species together. Some well-documented hybrid swarms exist, and others are suspected, but not all variable populations can be attributed to hybridization (Withner, 1974; Ackerman & Galarza-Pérez, 1991; Azevedo *et al.*, 2006; Jersáková *et al.*, 2006).

A second possibility is that periodic genetic drift (relaxed selection) may generate variation, particularly when population sizes are small, with even fewer reproductive individuals. Indeed many orchid populations do appear to be small, and fruit set is often low. Natural selection would have to be strong to overcome these conditions. There is some evidence consistent with this mechanism of generating unusually high levels of variation (Tremblay & Ackerman, 2001; Pellegrino *et al.*, 2007; Salzmann *et al.*, 2007). Genetic drift may also occur in larger populations when the driver of selection is no longer relevant, at least for a time. Although this has been demonstrated for some animals such as Darwin's Galapagos finches and suggested for plants (Grant & Grant 2006; Rivera-Marchand & Ackerman, 2006), we are not aware of a similar example in orchids.

The third possibility for unusually high levels of variation comes back to natural selection. In most cases it is expected that selection will reduce variation (Endler, 1986), but there are types of selection that can increase variation such as disruptive selection whereby unusual variants are those that have an advantage over common forms. Most flowering plants present a reward to their pollinators who search for food, materials for nest construction, and even resources to attract mates. These come in the form of nectar, pollen, resins, waxes, and even fragrances depending on the pollination system (Simpson & Neff, 1983). Thousands of orchid species, though, do not offer any pollinator reward whatsoever. These attract pollinators through deception by appearing to offer the resources that pollinators seek. Perhaps the most common form of deceit is the appearance as a food resource (Ackerman, 1986). Precise mimicry is not necessary as pollinators appear to be "wired" to recognize potential food sources. Bees, for example, test the availability of resources through exploratory visits soon after they emerge from their cells and later as their favored plants go out of flower. They visit a number of flowers of a certain type, and if they fail to extract a reward, then they will move to seek alternative resources (Heinrich, 1979). The idea is that if all flowers look the same, then the bees will learn quickly to avoid the species, but if flowers appear different, then it takes the bees longer to learn, and more flowers will be visited as a consequence (Heinrich, 1975). Not only would fruit set

be higher in variable populations, the bees would more likely move among plants after each failed exploratory visit resulting in an increase in the probability of cross-pollination (Johnson & Nilsson, 1999; Johnson *et al.*, 2004; Jersáková & Johnson, 2005). Thus, unusual variants would have the advantage, and their progeny would be better represented in the next generation.

The high levels of variation in deception-pollinated plants has been frequently commented upon, but there is only a single published report that compares population variation in deception and reward plants, and this was a single species pair of *Anacamptis* (Salzmann *et al.*, 2007). We do have ample unpublished data that indicate deception-pollinated plants are indeed generally more variable (Ackerman, Cuevas, and Hof, unpublished). However, the causes of such high levels of variation usually do not meet the expectations of negative frequency-dependent selection. In fact, only one case has been reported -- *Dactylorhiza sambucina* (L.) Soó -- and there is some dispute over that (Gigord *et al.*, 2001; Jersáková *et al.*, 2006). Thus far, all other cases studied (all using different methodologies) have failed to detect this type of selection and have indicated that relaxed selection or some other form of selection may be occurring (Ackerman *et al.*, 1997; Aragón & Ackerman, 2004; Ackerman & Carronero, 2005). This is not to say that natural selection, or even more specifically negative frequency-dependent selection, has not or will not occur but that only during the course of these particular studies it was not happening. Selection then is either difficult to detect in these plants or occurs for relatively brief periods of time as shown by the spasmodic diversification model (Tremblay *et al.*, 2005). Of course, there is yet another explanation: the methods for detecting selection were not rigorous enough or sufficiently sensitive to detect selection when it occurs.

Variation and plasticity

Variation is often presumed to be heritable, but the genetics of these traits are rarely examined in orchids, primarily because traditional methods require common garden experiments and crosses among the variants. For this, orchids are not good model systems because of the difficulty in propagating plants from seed (especially terrestrial species) and the relatively long period to first reproduction. Some plasticity in trait

expression occurs and is not likely directly inherited. For example, inflorescences of most species (not just orchids) have multiple flowers, and frequently the first flowers produced are larger than those that come later, a problem of inflorescence architecture and/or resource allocation (Tremblay, 2006; Herrera, 2009). Plastic responses may also occur in floral production after a pollination event (Ackerman, 1989; Harder and Johnson, 2005). We get around this problem by measuring all flowers of an inflorescence or just the lowermost flower. However, plasticity also occurs in response to environmental heterogeneity. Microhabitat gradients occur within populations, and plants respond accordingly. Environmental conditions can also vary from year to year as habitats go through successional processes or as local climate changes, whether temporary (El Niño or La Niña) or relatively permanent (local urbanization or global changes). Phenotypic responses for given genotypes are well known among plants (Pigliucci, 2001), yet plasticity in orchids has received scant attention. Should plastic versions of traits result in differential fitness, then detecting selection within a population may become additionally problematic. Certainly phenotypic plasticity can blunt the power of selection.

Plasticity in orchids: a test case

How much plasticity occurs in orchid morphology, and how is this related to fitness? We addressed this question in a recent paper (Morales *et al.*, 2010) by studying a population of *Tolumnia variegata* (Sw.) Braem, a deception-pollinated twig epiphyte occurring in the Greater Antilles from the Virgin Islands in the east to western Cuba. This is a species for which attempts were made to detect negative frequency-dependent selection but without success (Ackerman *et al.*, 1997). In fact, no selection of any kind on any floral characteristic (including fragrance production) was revealed, except for disruptive selection on flowering phenology and weak directional selection on the number of flowers (Sabat and Ackerman, 1996).

Large populations of *T. variegata* are not uncommon and can be found from dry forests to wet montane regions (Ackerman, in press). Plants are small with sympodial growth. Morphological and genetic diversity are high (Fig. 1). Genetic differentiation among populations is low, and estimates of gene flow



FIGURE 1. Variation in flowers from a single population of *Tolumnia variegata* in Puerto Rico. Each flower came from a different plant. Photo: James D. Ackerman.

are high, particularly among populations of a given island (Ackerman and Galarza-Pérez, 1991; Ackerman and Ward, 1999). Flowers are self-incompatible, and pollinators are female *Centris decolorata* (Apidae; Ackerman *et al.*, 1997), presumably deceived by flowers appearing as an oil resource.

To induce plastic responses, we conducted a reciprocal transplant experiment where our environmental gradient of interest was light. Plants grew in the open under full sun and also in the shade. We used 80 plants from two sun sites and 80 plants from two shady sites, all of which were within a 25 m radius. Prior to transplanting the orchids, we found that shade plants had larger leaves and longer inflorescences but fewer flowers than sun plants. After transplanting, we followed these plants for two years and recorded a number of vegetative, inflorescence, and floral characteristics on each plant and monitored female reproductive success (fruit production). The first year after transplanting (1999) was a wet one, about 200 mm above normal precipitation. The second year (2000) was a dry one, with precipitation approximately 500 mm below normal.

Using repeated measures ANOVA to analyze results of the transplant experiment, we found that plant responses were complex (Morales *et al.*, 2010). Leaf characteristics of plants transplanted from sun to shade over time looked much like those that originated and stayed in the shade. Likewise, those that went from shade to sun became more like sun plants. Leaf size in general was strongly affected by drought conditions in the second year.

Measures of reproductive effort gave mixed results. The number of flowers did not change. Peduncle length was strongly affected by year, whereas the number of inflorescences was more affected by environmental variation.

Floral characteristics we measured were associated with floral display, not with pollination mechanics. All appeared to be plastic, and all were affected by year. Origin of transplants had a significant effect in three of five measures, and flowers in the shade were larger in three of five measures as well.

How did transplants differ in reproductive success? Surprisingly, fruit set was not affected by environment, even though there were significant differences in plant traits between the sun and shade sites. We did not measure male reproductive success where all the action may occur, as has been shown in other orchid systems (Ackerman & Carronero, 2005).

Because the picture thus far is clear only in the sense that it is muddy, we decided to take an alternative approach to detecting patterns of selection by using the non-parametric, cubic spline regression analysis (see Tremblay *et al.*, 2010). We used data for only those characteristics that showed significant results in the repeated measures ANOVAs. We found that for petal width the overall pattern was for disruptive selection, and this was also reflected in the individual patterns for shade and sun plants. Thus, the two habitats behaved the same with respect to this character. For flower number, there was an overall trend for larger inflorescences to have greater fitness, but this was mostly limited to sun plants because shade plants showed no trend whatsoever. Regardless, this trend is what was detected in an earlier study at the same locality (Sabat and Ackerman, 1996). Finally, we compared lip length and found a negative trend. However, when shade plants and sun plants were analyzed separately, we found that shade plants have a slight positive trend, whereas sun plants show a strong negative trend affecting the overall selection pattern.

When selection across microhabitats is consistent, then evolution has the potential to be fast. But when selection patterns across an environmental gradient are on different trajectories, then the speed of change may be gradual or nil, making it difficult to detect at the population level. Such conflicts may occur across habitats as well as over time.

Conclusions

The difficulty in detecting selection in orchid populations may be explained by the possibility that selection is not occurring, or as we discussed here, selection may go in different directions depending on where they are in a heterogeneous habitat. The picture can get even more opaque when one considers phenotypic responses over a number of seasons when environmental conditions change from year to year. Using a Bayesian approach with data from this *Tolumnia* study plus data from a population of *Caladenia*, Tremblay *et al.* (2010) found that indeed selection trajectories can vary not only among habitats but also among years, and significantly so. Thus we find yet another reason why selection may be difficult to detect in orchids. The notion that selection need not be operational all the time should come as no surprise. Consequently, we find that these studies are consistent with the spasmodic model of orchid diversification whereby periods of drift (no selection) may be punctuated by brief but strong bouts of selection (Tremblay *et al.*, 2005).

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HOW MANY ORCHID SPECIES IN COSTA RICA? A REVIEW OF THE LATEST DISCOVERIES

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ABSTRACT. Abstract. Despite its well-established tradition in botanical exploration, which started in 1846 with the visit of Oersted (1846), Costa Rica is still far from having a complete inventory of its orchidaceous flora. After the publication of the most recent and complete treatment of the family by Dressler in 2003, new species and records have been added on a regular basis to the country's inventory. Showy, large-flowered species in previously monographed and botanically well-sampled genera such as *Brassia*, *Dracula*, *Lycaste*, *Polycycnis*, *Stanhopea*, and *Trichopilia* have been described, but the vast majority of species are small-flowered and belong to the subtribes Laeliinae, Pleurothallidinae, and Zygopetalinae. Identifying taxa with ephemeral flowers such as *Sobralia* is problematic, but a large living collection revealed many new species. Previously described species from other countries have regularly been recorded in Costa Rica. These new records have floristic affinities mainly with the floras of Panama, Colombia, Ecuador, and Venezuela. As an example, *Acianthera aberrans*, *Epidendrum scharfii*, *Epidendrum stellidiforme*, *Lockhartia chocoensis*, *Maxillaria bolivarensis*, *Ornithidium pendulum*, *Ornithocephalus montealegrae*, and *Warmingia zamorana* have been found in both Costa Rica and Ecuador. The genus *Uleiorchis* with the Venezuelan species *Uleiorchis ulaei*, identified in the MO herbarium by Ron Liesner, constitutes an interesting new record in Costa Rica. *Maxillaria appendiculoides*, first described from Costa Rica, has recently been reported from Ecuador. *Campylocentrum tenellum*, *Lepanthes droseroides*, *Lepanthes mariposa*, and *Sobralia bouchei* from Panama were also lately collected in Costa Rica. Although much floristic work remains to be completed and the country has significant areas that are poorly sampled, the establishment of large and documented collections of living plants at Lankester Botanical Garden, associated with an increasing access to critical documentation (types and literature), have been the keys to improving our understanding of orchid diversity in Costa Rica and its floristic relationships with other areas.

Resumen. A pesar de su bien establecida tradición en exploración botánica, la cual comenzó en 1846 con la visita de Oersted (1846), Costa Rica está todavía lejos de tener un inventario completo de su flora de orquídeas. Después de la publicación del tratamiento más reciente y completo de la familia por Dressler (2003) nuevas especies y registros han sido añadidos regularmente al inventario del país. Especies llamativas, de flores grandes han sido descritas en géneros previamente monografiados y botánicamente bien muestreados, tales como: *Brassia*, *Dracula*, *Lycaste*, *Polycycnis*, *Stanhopea*, y *Trichopilia*, pero la gran mayoría de especies son plantas de flores pequeñas que pertenecen a las subtribus Laeliinae, Pleurothallidinae, y Zygopetalinae. Las flores efímeras, como *Sobralia* tienen problemas especiales con su identificación pero una colección viva, grande, revela muchas nuevas especies. Las especies previamente descritas de otros países han sido registradas en Costa Rica con regularidad. Estos nuevos registros tienen afinidades florísticas principalmente con la flora de Panamá, Colombia, Ecuador, y Venezuela. Por ejemplo, *Acianthera aberrans*, *Epidendrum scharfii*, *Epidendrum stellidiforme*, *Lockhartia chocoensis*, *Maxillaria bolivarensis*, *Ornithidium pendulum*, *Ornithocephalus montealegrae*, y *Warmingia zamorana* han sido encontradas tanto en Costa Rica como en Ecuador. El género *Uleiorchis* con la especie venezolana *Uleiorchis liesneri*, identificado en el herbario MO por Ron Liesner, constituye un nuevo registro interesante en Costa Rica. También, *Maxillaria appendiculoides* descrita de Costa Rica, ha sido registrada recientemente en Ecuador. *Campylocentrum tenellum*, *Lepanthes droseroides*, *Lepanthes mariposa*, y *Sobralia bouchei* de Panamá también fueron colectadas recientemente en Costa Rica. Aunque mucho trabajo florístico queda por ser completado y el país tiene áreas significativas que

están mal muestreadas, el establecimiento de colecciones grandes y documentadas de plantas vivas en el Jardín Botánico Lankester, asociado con un acceso creciente a la documentación crítica (tipos y literatura), han sido la llave para el mejoramiento de nuestro entendimiento de la diversidad de orquídeas en Costa Rica y sus relaciones florísticas con otras áreas.

KEY WORDS: Orchidaceae, Costa Rica, novelties

Despite its well-established tradition in botanical exploration, which started in 1846 with the visit of Oersted (1846), Costa Rica is still far from having a complete inventory of its orchidaceous flora (Bogarín *et al.*, 2008). During the second half of the 19th century Hemsley (1883) and Reichenbach (1886) published the first inventories for the orchids of Mesoamerica. Later, Schlechter (1918), Ames (in Standley, 1937), and Williams (1956) updated the species' list for Central America. The need for an up-to-date checklist of the Orchidaceae in Costa Rica was filled in part by Mora-Retana and García (1992), Pupulin (2002), and Dressler (1993; 2003). More recently, Ossenbach *et al.* (2007) published a checklist of the orchids of Costa Rica and Mesoamerica.

Throughout the history of documentation of the Orchidaceae in Costa Rica, more than three quarters of the species were named by botanists who deposited the material outside the country. Although botanical exploration began almost 150 years ago, the documentation of the orchid flora was developed sporadically by botanists during the past 15 decades. In the 19th century, Bateman, Lindley, Reichenbach, and Rolfe named the first species known for the country. Orchid research declined from 1860 to 1900 until Ames and Schlechter worked intensively on the Neotropical Orchidaceae (Fig. 1). However, the number of species named declined dramatically between 1950 and 1960, when less than 30 species were described for Costa Rica, contrasting with the decade of 1920 when nearly 300 of the species were named (Fig. 1). Even though there was botanical exploration in those years, the botanists never managed to document permanently the flora of Costa Rica. This was mainly attributable to the lack of critical materials to identify the species and also the fact that the literature was often published as a few copies that circulated only in developed countries.

The Global Taxonomy Initiative (GTI) of the Convention on Biological Diversity (CBD) pointed out a critical problem for effective conservation management that largely depends on taxonomic

knowledge: “the inadequate taxonomic information and infrastructure, coupled with declining taxonomic expertise.” Unfortunately, one of the main problems developing taxonomic expertise in tropical countries has been the lack of adequate information for species identification. The existence of this taxonomic impediment is the major problem to solve by global strategies such as GTI. With the establishment of Lankester Botanical Garden (LBG) in 1973 and other institutions such as Museo Nacional de Costa Rica and Instituto Nacional de Biodiversidad (INBio) there has been an increase in orchid research that has continued until the present. It has been demonstrated that LBG has played a major role in revealing orchid diversity through a well-developed taxonomic system (Fig. 1). The mission of LBG has been to eliminate the taxonomic impediment through gathering critical information and making it available for researchers and students.

Floristics and botanical exploration in Costa Rica are the basis for future research and conservation actions. New techniques for species identification using DNA sequences or DNA barcoding have been developed; however, a well-established taxonomic system is needed to solve the problem of incomplete floristic inventories and for developing new techniques that

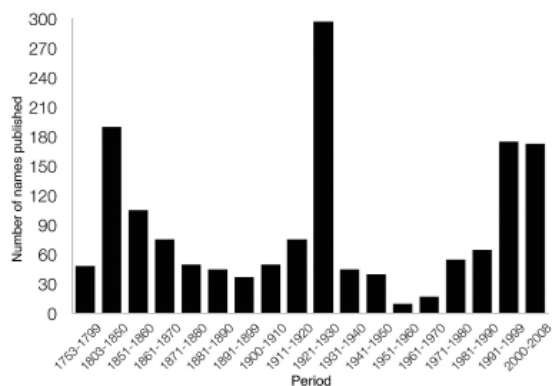


FIGURE 1. Comparison of the number of species of orchids described from Costa Rica at different periods.

will not work without an adequate taxonomic basis (Lahaye *et al.*, 2008). The aim of the present paper is to discuss the status of the Orchidaceae in Costa Rica, where new species are continuously being discovered.

A review of the latest discoveries

After the publication of the most complete treatment of the Orchidaceae from Costa Rica by Dressler (2003), new species and new records have been added on a regular basis to the country's orchid checklist. Ossenbach *et al.* (2007) estimated that 1461 orchid species occur in Costa Rica. After Ossenbach *et al.* (2007), 34 species were described as new to science and 24 species previously described outside Costa Rica were recorded for this country. The number now stands at 1519 species. A summary of the latest discoveries and a list of additional material of several species known from a single locality or specimen are provided in Table 1 with synonyms, authorities, and voucher information. Endemic species are annotated there with an asterisk.

Several species recorded by Ossenbach *et al.* (2007) are listed here with their respective voucher. Among the species cited, 29 are endemic to Costa Rica. There are 25 species found both in Costa Rica and Panama, and 14 shared with Ecuador. Some disjunctions are recorded, such as *Epidendrum stellidiforme* and *Warmingia zamorana*, which are known only from Costa Rica and Ecuador, but their presence in Panama and Colombia is highly probable (Bogarín *et al.*, 2008). Also, there are 16 species widespread from Costa Rica to South America and 10 ranging from Costa Rica to Nicaragua, Honduras, Guatemala or Mexico.

Subtribe Angraecinae: *Campylocentrum tenellum* Todzia is recorded for a first time in Costa Rica. Two specimens of *C. tenellum* from different localities in Costa Rica are known, but unfortunately in sterile condition. A recent fertile collection provided a proper recording of this species (Fig. 2a, 3c). An aphyllous species with flat roots was recorded from El Valle del General on the Pacific watershed of the Cordillera de Talamanca. Although *C. pachyrrhizum* (Rchb.f.) Rolfe is expected to occur in Costa Rica, the material collected indicates that it may correspond to an undescribed species. *Campylocentrum multiflorum* Schltr. is accepted and considered as distinct from *C.*

fasciola (Lindl.) Cogn. The name *C. tyrridion* Garay & Dunst. should be best applied to the species from Venezuela. The Costa Rican voucher of *C. dressleri* H.Dietr. & M.A.Díaz (*J. Villalobos s.n.* (USJ!)) clearly corresponds to *C. multiflorum* Schltr., so *C. dressleri* is excluded from Costa Rica (Bogarín and Pupulin, 2009).

Tribe Gastrodieae: An interesting record of the holomycotrophic orchid genus *Uleiorchis* is *U. ulaei* (Cogn.) Handro, which was recently identified by Ron Liesner at MO. The species had been recorded in Honduras, Panama, and South America.

Subtribe Goodyerinae: Studies published by Ormerod (2007, 2008) revealed a new species of *Aspidogyne*, *A. grayumii* Ormerod, known from the Estación Biológica Las Cruces in southern Costa Rica near Panama. A new *Kreodanthus*, *K. curvatus* Ormerod, is known from the northwest slope of Volcán Barva in the Central Cordillera and also from a collection in the Cordillera de Talamanca. It is compared with the Panamanian *K. bugabae* Ormerod. Two new species of *Microchilus* were also recorded — *M. maasii* Ormerod from the Monteverde region (also from central Panama) and *M. tessellatus* Ormerod, endemic to Costa Rica. The recently described *M. valverdei* Ormerod is now a synonym of *M. calophyllus* (Rchb.f.) Ormerod. Also, *Platythelys alajuelae* Ormerod is endemic to Costa Rica and known from the Cordillera de Tilarán and the Cordillera Central.

Subtribe Laeliinae: Preliminary results of a revision of *Encyclia* in Costa Rica yielded a new record: *Encyclia gravida* (Lindl.) Schltr. Plants of this species are usually recognized by having cleistogamous flowers. The number of new species and records is considerable in the large genus *Epidendrum*. The additions have been mainly published by Hágsater and co-workers (2004, 2006, 2008). The following new species have been described: *E. apatotylosum* Hágsater, *E. astro-selaginellum* Hágsater & E.Santiago, *E. bicuniculatum* Hágsater & E.Santiago, *E. isthmoides* Hágsater & E.Santiago (recorded recently; Fig. 6d), *E. xnocteburneum* Hágsater & L.Sánchez (a natural hybrid between *E. eburneum* Rchb.f. and *E. nocturnum* Jacq.), *E. philowercklei* Hágsater & E.Santiago, *E. salpichlamys* Hágsater & E.Santiago, *E. steno-*

TABLE 1. List of recent additions to the orchid flora of Costa Rica

Species	Voucher
1. <i>Acianthera aberrans</i> (Luer) Pupulin & Bogarín, Lankesteriana 8(2): 53-55. 2008.	Pupulin 4857, JBL
2. <i>Acianthera cabiriae</i> Pupulin, G.A.Rojas & J.D.Zuñiga, Harvard Pap. Bot. 12(1): 160. 2007. *	Karremans 1433, CR
3. <i>Acianthera fecunda</i> Pupulin, G.A.Rojas & J.D.Zuñiga, Harvard Pap. Bot. 12(1): 158. 2007. *	Bogarín 2650, CR
4. <i>Acianthera hamata</i> Pupulin & G.A.Rojas, Harvard Pap. Bot. 12(1): 156. 2007. *	Quesada Chanto s.n., CR
5. <i>Aspidogyne grayumii</i> Ormerod, Harvard Pap. Bot. 11(2): 147. 2007. *	Grayum 9267, MO
6. <i>Barbosella orbicularis</i> Luer, Selbyana 3(1,2): 10-11, f. 108. 1976.	Bogarín 1949, JBL
7. <i>Bensteinia ramonensis</i> Pupulin, Selbyana 28(2): 113. 2008. *	Bogarín 1923, CR
8. <i>Campylocentrum tenellum</i> Todzia, Ann. Missouri Bot. Gard. 72: 877. 1985.	Bogarín 5844, JBL
9. <i>Dichaea gomez-lauritoi</i> Pupulin, Harvard Pap. Bot. 12(1): 87. 2007. *	Gómez-Laurito 8174, CR
10. <i>Dryadella fuchsii</i> Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 162-163. 1999.	Standley & Valerio 45606, AMES
11. <i>Echinosepala lappiformis</i> (A.H.Heller & L.O.Williams) Pridgeon & M.W.Chase Lindleyana 17(2): 101. 2002. <i>Pleurothallis lappiformis</i> A.H.Heller & L.O.Williams, Fieldiana, Bot. 31(2): 42, f. 8. 1964. <i>Myoxanthus lappiformis</i> (A.H.Heller & L.O.Williams) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 15: 38. 1986. <i>Brenesia lappiformis</i> (A.Heller & L.O.Williams) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 255. 2004. <i>Echinella lappiformis</i> (A.Heller & L.O.Williams) Pridgeon & M.W.Chase, Lindleyana 16(4): 253. 2001.	Dressler 6768, JBL
12. <i>Elleanthus ligularis</i> Dressler & Bogarín, Lankesteriana 7(3): 539. 2007.	Dressler 6836B, CR
13. <i>Encyclia gravida</i> (Lindl.) Schltr., Beih. Bot. Centralbl. 36(2): 472. 1918. <i>Epidendrum gravidum</i> Lindl., J. Hort. Soc. London 4: 114. 1849.	Pupulin 5377, JBL
14. <i>Epidendrum acroamparoanum</i> Hágsater & L.Sánchez S., Icon. Orchid. (Mexico) 8: t. 802. 2006. *	Morales 4737, MO
15. <i>Epidendrum atypicum</i> Hágsater & E. Santiago A., Icon. Orchid. (Mexico) 8: t. 810. 2006. *	Weber 6081, AMES
16. <i>Epidendrum angustilobum</i> Fawc. & Rendle, J. Bot. 47(556): 124-125. 1909. <i>Epidendrum latifolium</i> (Lindl.) Garay & H.R. Sweet, J. Arnold Arbor. 53(3): 392. 1972.	Moraga 312, INB
17. <i>Epidendrum apatotylosum</i> Hágsater, Icon. Orchid. 9: t. 909. 2007. *	Horich sub E. Hágsater 7117, INB
18. <i>Epidendrum arcuiflorum</i> Ames & C.Schweinf., Schedul. Orch. 10: 58-59. 1930. <i>Epidendrum powellii</i> Schltr., Repert. Spec. Nov. Regni Veg. Beih. 17: 38. 1922.	Herrera 3595, CR
19. <i>Epidendrum astroselaginellum</i> Hágsater & E.Santiago, Icon. Orchid. 9: t. 910. 2007.	Gómez 23814, MO
20. <i>Epidendrum bicuniculatum</i> Hágsater & E.Santiago, Icon. Orchid. 9: t. 913. 2007. *	Grayum & G. Herrera 7716, MO
21. <i>Epidendrum buenaventurae</i> F.Lehm. & Kränzl., Bot. Jahrb. Syst. 26(5): 470-471. 1899.	Gómez 19984, MO
22. <i>Epidendrum chalcochromum</i> Hágsater, Icon. Orchid. (Mexico) 8: t. 818. 2006. *	Hágsater & Mora de Retana 9495, INB

Species	Voucher
23. <i>Epidendrum cristatum</i> Ruiz & Pav., Syst. Veg. Fl. Peruv. Chil. 1: 243. 1798. <i>Epidendrum raniferum</i> Lindl., Gen. Sp. Orchid. Pl.109. 1831.	<i>Brade 1281</i> , AMES
24. <i>Epidendrum dolichoclamys</i> Hágsater & E.Santiago A., Icon. Orchid. (Mexico) 8: t. 829. 2006. *	<i>Chavarría 661</i> , INB
25. <i>Epidendrum gibbosum</i> L.O.Williams, Ann. Missouri Bot. Gard. 28(4): 420-421, pl. 21, 7-10. 1941. <i>Epidendrum acrostigma</i> Hágsater & García-Cruz, Icon. Orchid. 3: t. 301. 1999.	<i>Grayum 4578</i> , CR
26. <i>Epidendrum haberi</i> Hágsater & L.Sánchez S., Icon. Orchid. (Mexico) 8: t. 840. 2006. *	<i>Haber & Cruz 7245</i> , INB
27. <i>Epidendrum hawkesii</i> A.H.Heller, Phytologia 14(1): 2-4, t. 1. 1966.	<i>Horch sub Hágsater 7649</i> , AMO
28. <i>Epidendrum isthmoides</i> Hágsater & E.Santiago, Icon. Orchid. 11: t. 1133. 2008. *	<i>Bello 1516</i> , INB
29. <i>Epidendrum maduroi</i> Hágsater & García-Cruz, Icon. Orchid. (Mexico) 3: pl. 352. 1999.	<i>Bogarín 1465</i> , JBL
30. <i>Epidendrum microrigidiflorum</i> Hágsater, Icon. Orchid. (Mexico) 8: t. 853. 2006.	<i>Dressler 6259</i> , AMO
31. <i>Epidendrum nocteburneum</i> Hágsater & L.Sánchez, Icon. Orchid. 11: t. 1148. 2008.	<i>Weston 57 sub Rodríguez 1042</i> , USJ
32. <i>Epidendrum orthodontum</i> Hágsater & L.Sánchez, Icon. Orchid. 3: t. 361. 1999.	<i>Karremans 2255</i> , JBL
33. <i>Epidendrum pachyepalum</i> Hágsater & E.Santiago A., Icon. Orchid. (Mexico) 8: t. 865. 2006.	<i>Burger 8253</i> , F
34. <i>Epidendrum pendens</i> L.O.Williams, Ann. Missouri Bot. Gard. 28(4): 421-422, pl. 23. 1941.	<i>Haber & Cruz 7986</i> , CR
35. <i>Epidendrum philowercklei</i> Hágsater & E.Santiago A., Icon. Orchid. (Mexico) 8: t. 870. 2006.	<i>Fernández 677</i> , CR
36. <i>Epidendrum platystomoides</i> Hágsater & L.Sánchez S., Icon. Orchid. (Mexico) 8: t. 872. 2006. *	<i>Hágsater & Mora de Retana 9507</i> , AMO
37. <i>Epidendrum purpurascens</i> H.Focke, Tijdschr. Wis- Natuurk. Wetensch. Eerste Kl. Kon. Ned. Inst. Wetensch.) 4: 64-65. 1851. <i>Epidendrum glumibracteum</i> Rchb.f., Hamburger Garten- Blumenzeitung 19: 11. 1863.	<i>Valerio 1017</i> , CR
38. <i>Epidendrum rousseauae</i> Schltr., Beih. Bot. Centralbl., Abt. 2 36(2): 407-408. 1918.	<i>Todzia 552</i> , CR
39. <i>Epidendrum salpichlamys</i> Hágsater & E.Santiago A., Icon. Orchid. (Mexico) 8: t. 883. 2006. *	<i>Grayum & Pam Sleeper 3446</i> , MO
40. <i>Epidendrum scharfii</i> Hágsater & Dodson, Icon. Orchid. 2: t. 185. 1993.	<i>Pupulin 6500</i> , JBL
41. <i>Epidendrum serruliferum</i> Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 44. 1923. *	<i>Wercklé 114</i> , B, destroyed; drawing of type, AMES
42. <i>Epidendrum stellidiforme</i> Hágsater & Dodson, Icon. Orchid. (Mexico) 4: t. 487. 2001.	<i>Bogarín 2814</i> , JBL
43. <i>Epidendrum stenoselaginellum</i> Hágsater & E.Santiago, Icon. Orchid. 9: t. 991. 2007.	<i>Hágsater 11115</i> , AMO
44. <i>Epidendrum tritropianthum</i> Hágsater & E.Santiago A., Icon. Orchid. (Mexico) 9(6): t. 998. 2007.	<i>Ingram & Ferrell 743</i> , INB
45. <i>Epidendrum villotae</i> Hágsater & Dodson, Icon. Orchid. 3: pl. 398. 1999.	<i>Herrera 5151</i> , MO

Species	Voucher
46. <i>Epidendrum vulcanicola</i> A.H.Heller, Fieldiana, Bot. 32(2): 11, f. 2. 1968.	Alcázar 56, USJ
47. <i>Epidendrum vulgoamparouanum</i> Hágsater & L.Sánchez S., Icon. Orchid. (Mexico) 8: t. 898. 2006.	Hágsater 6963, INB
48. <i>Epidendrum zunigae</i> Hágsater, Karremans & Bogarín, Lankesteriana 8(2): 63. 2008. *	Bogarín 2680, JBL
49. <i>Gongora boracayanensis</i> Jenny, Dalström & W.E.Higgins, Selbyana 28(2): 99. 2007. *	Clark et al. 314 B, SEL
50. <i>Kefersteinia alata</i> Pupulin, Harvard Pap. Bot. 8(2): 161-164, f. 1, 2A-B. 2004.	Carman s.n., JBL
51. <i>Kefersteinia saccata</i> Pupulin, Willdenowia 38(1): 188. 2008. *	Pupulin 6549, CR
52. <i>Kreodanthus curvatus</i> Ormerod, Harvard Pap. Bot. 13(1): 61. 2008. *	Grayum 7481, MO
53. <i>Lepanthes droseroides</i> Luer, Lindleyana 2: 188. 1987.	Bogarín 5234, JBL
54. <i>Lepanthes gratiosa</i> Pupulin & D.Jiménez, Orchid Digest 73: 137-139. 2009. *	Pupulin 7350, CR
55. <i>Lepanthes machogaffensis</i> Pupulin & D. Jiménez, Orchid Digest 73: 139-140. 2009. *	Pupulin 7308, CR
56. <i>Lepanthes mariposa</i> Luer, Phytologia 55: 187. 1984.	Bogarín 5034, JBL
57. <i>Lepanthes pelvis</i> Pupulin & D.Jiménez, Orchid Digest 73: 142-144. 2009. *	Pupulin 7336, CR
58. <i>Lockhartia chocöensis</i> Kränzl. in H.G.A.Engler (ed.), Pflanzenr., IV, 50(83): 19. 1923.	Bogarín 2352, JBL
59. <i>Lycaste angelae</i> Oakeley, Lycaste, Ida, Anguloa 27. 2008.	Oakeley A5 (CR7), K-HFO
60. <i>Lycaste bruncana</i> Bogarín, Lankesteriana 7(3): 543. 2007. <i>Lycaste crystallina</i> Wubben ex Oakeley, Lycaste, Ida, Anguloa 73. 2008. *	Bogarín 3987, CR
61. <i>Lycaste x daniloi</i> Oakeley, Lycaste, Ida, Anguloa 76. 2008.	Oakeley 14 (CR14), K-HFO
62. <i>Lycaste panamanensis</i> (Fowlie) Oakeley, Orchid Digest 71: 205. 2007. <i>Lycaste macrophylla</i> subsp. <i>panamanensis</i> Fowlie, Lasca Leaves 14(1): 40, f. 1964.	see Oakeley (2008)
63. <i>Maxillaria bolivarensis</i> C.Schweinf., Bot. Mus. Leafl. 20: 22. 1962.	Whitten 2030, JBL
64. <i>Microchilus maasii</i> Ormerod, Harvard Pap. Bot. 11(2): 161. 2007.	Bello 2809, CR
65. <i>Microchilus tessellatus</i> Ormerod, Harvard Pap. Bot. 11(2): 172. 2007. *	Grayum 8925, MO
66. <i>Ornithidium pendulum</i> (Poepp. & Endl.) Cogn., Fl. Bras.3(6): 92. 1904.	Karremans 448, JBL
67. <i>Palmorchis nitida</i> Dressler, Die Orchidee 34(1): 29-30. 1983.	Gómez 26248, USJ
68. <i>Platythelys alajuelae</i> Ormerod, Harvard Pap. Bot. 11(2): 174. 2007. *	Ingram & Ferrell 680, MO
69. <i>Pleurothallis bogarinii</i> Pupulin & J.D.Zuñiga, Orchids 76 (Lindleyana): 690. 2007. *	Pupulin 5285, CR
70. <i>Pleurothallis caudatipetala</i> C.Schweinf., Bot. Mus. Leafl.10: 175, t. 22, f. 5-8. 1942. <i>Specklinia caudatipetala</i> (C. Schweinf.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard.95: 259. 2004. <i>Panmorphia caudatipetala</i> (C. Schweinf.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard.105: 150. 2006.	Luer 12137, MO
71. <i>Pleurothallis duplooyi</i> Luer & Sayers, Revista Soc. Boliv. Bot.3(1/2): 48-50. 2001. <i>Specklinia duplooyi</i> (Luer & Sayers) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 260. 2004. <i>Panmorphia duplooyi</i> (Luer & Sayers) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 153. 2006.	Bogarín 6955, JBL

Species	Voucher
72. <i>Pleurothallis minutalis</i> Lindl., Fol. Orchid. Pleurothallis 40. 1859. <i>Humboldtia minutalis</i> (Lindl.) Kuntze, Revis. Gen. Pl.2: 668. 1891. <i>Humboldtia minutalis</i> (Lindl.) Kuntze, Revis. Gen. Pl.2: 668. 1891. <i>Panmorphia minutalis</i> (Lindl.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard.105: 167. 2006. <i>Specklinia minutalis</i> (Lindl.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004. <i>Anathallis minutalis</i> (Lindl.) Pridgeon & M.W. Chase, Lindleyana 16(4): 249. 2001.	Endrés s.n., W
73. <i>Restrepiella lueri</i> Pupulin & Bogarín, Willdenowia 37(1): 325. 2007. *	Bogarín 3009, CR
74. <i>Scaphyglottis robusta</i> B.R.Adams, Phytologia 64: 253. 1988.	Bogarín 2662, JBL
75. <i>Sobralia aspera</i> Dressler & Pupulin, Orquideología 25(2): 148. 2008. *	Dressler 6783, CR
76. <i>Sobralia blancoi</i> Dressler & Pupulin, Orquideología 25(2): 139. 2008. *	Dressler 6706, CR
77. <i>Sobralia bouchei</i> Ames & C.Schweinf., Schedul. Orchid. 10: 4. 1930.	Bogarín 4201, JBL
78. <i>Sobralia pendula</i> Dressler & Pupulin, Orquideología 25(2): 140. 2008. *	Dressler 6746, CR
79. <i>Sobralia rarae-avis</i> Dressler, Orquideología 25(1): 38. 2007. *	Dressler 6793, CR
80. <i>Trichopilia primulina</i> Dressler & Bogarín, Orchideen J. 16(2): 60. 2009. *	Dressler 7030, CR
81. <i>Uleiorchis ulaei</i> (Cogn.) Handro, Arq. Bot. Estado São Paulo 3(4): 175. 1958. <i>Wulschlaegelia ulaei</i> Cogn., Fl. Bras. 3(4): 244. 1895.	Hammel 11657, MO
82. <i>Warmingia zamorana</i> Dodson, Icon. Pl. Trop., II, 6: t. 599. 1989. <i>Warmingia margaritacea</i> B.Johans., Lindleyana 7: 194. 1992.	Karremans 1123, JBL

selaginellum Hágsater & E.Santiago (from Nicaragua, Costa Rica, and Panama), and *E. zunigae* Hágsater, Karremans & Bogarín (Fig. 4g, 5d). The following new species were described by Hágsater *et al.* (2006) and recorded by Ossenbach *et al.* (2007): *Epidendrum acroamparoanum* Hágsater & L.Sánchez, *E. atypicum* Hágsater & E.Santiago, *E. chalcochromum* Hágsater, *E. dolicho-chlamys* Hágsater & E.Santiago, *E. haberi* Hágsater & L.Sánchez, *E. microrigidiflorum* Hágsater, *E. pachytepalum* Hágsater & E. Santiago, *E. philowercklei* Hágsater & E. Santiago, *E. platystomoides* Hágsater & L.Sánchez, *E. salpichlamys* Hágsater & E.Santiago, *E. tritropianthum* Hágsater & E.Santiago, and *E. vulgoamparoanum* Hágsater & L.Sánchez (previously known as *E. amparoanum* Schltr., which is now treated as a synonym of *E. barbeyanum* Kränzl.). *Epidendrum microrigidiflorum*, *E. pachytepalum*, *E. philowercklei*, and *E. vulgoamparoanum* are found in both Costa Rica and Panama. New collections of previously described species have been added regularly: *E. buenaventurae* F.Lehm. & Kränzl., *E. scharfii* Hágsater & Dodson (Fig. 4f), *E. stellidiforme* Hágsater & Dodson (Fig. 4e, 5c), and *E. villotae* Hágsater & Dodson, all recorded previously from

Ecuador; *Epidendrum hawkesii* A.H.Heller and *E. vulcanicola* A.H.Heller from Nicaragua; and *E. maduroi* Hágsater & García-Cruz (Fig. 4d), *Epidendrum orthodontum* Hágsater & L.Sánchez (Fig. 5b), *E. pendens* L.O.Williams, and *E. rousseauae* Schltr. from Panama, which was formerly listed by Pupulin (2002). The following taxonomic changes affecting Costa Rican species have been noted by Hágsater (2008): *Epidendrum angustilobum* Fawc. & Rendle (= *E. latifolium* (Lindl.) Garay & H.R.Sweet), *E. arcuiflorum* Ames & C.Schweinf. (= *E. powellii* Schltr.), *E. cristatum* Ruiz & Pav. (= *E. raniferum* Lindl.), *E. gibbosum* L.O.Williams (= *E. acrostigma* Hágsater & García-Cruz), *E. purpurascens* H.Focke (= *E. glumibracteum* Rehb.f.), and the reconsidered *E. serruliferum* Schltr. Two new species and a new addition will be soon published by Pupulin and Karremans (unpublished). A collection of *Scaphyglottis robusta* B.R.Adams, previously described from Panama, has been documented in tropical wet premontane forest at an elevation of 1150 m in the Caribbean watershed of the Cordillera de Talamanca (Fig. 10b). Another specimen was collected in the region of Turrialba in the province of Cartago (Fig. 4j).

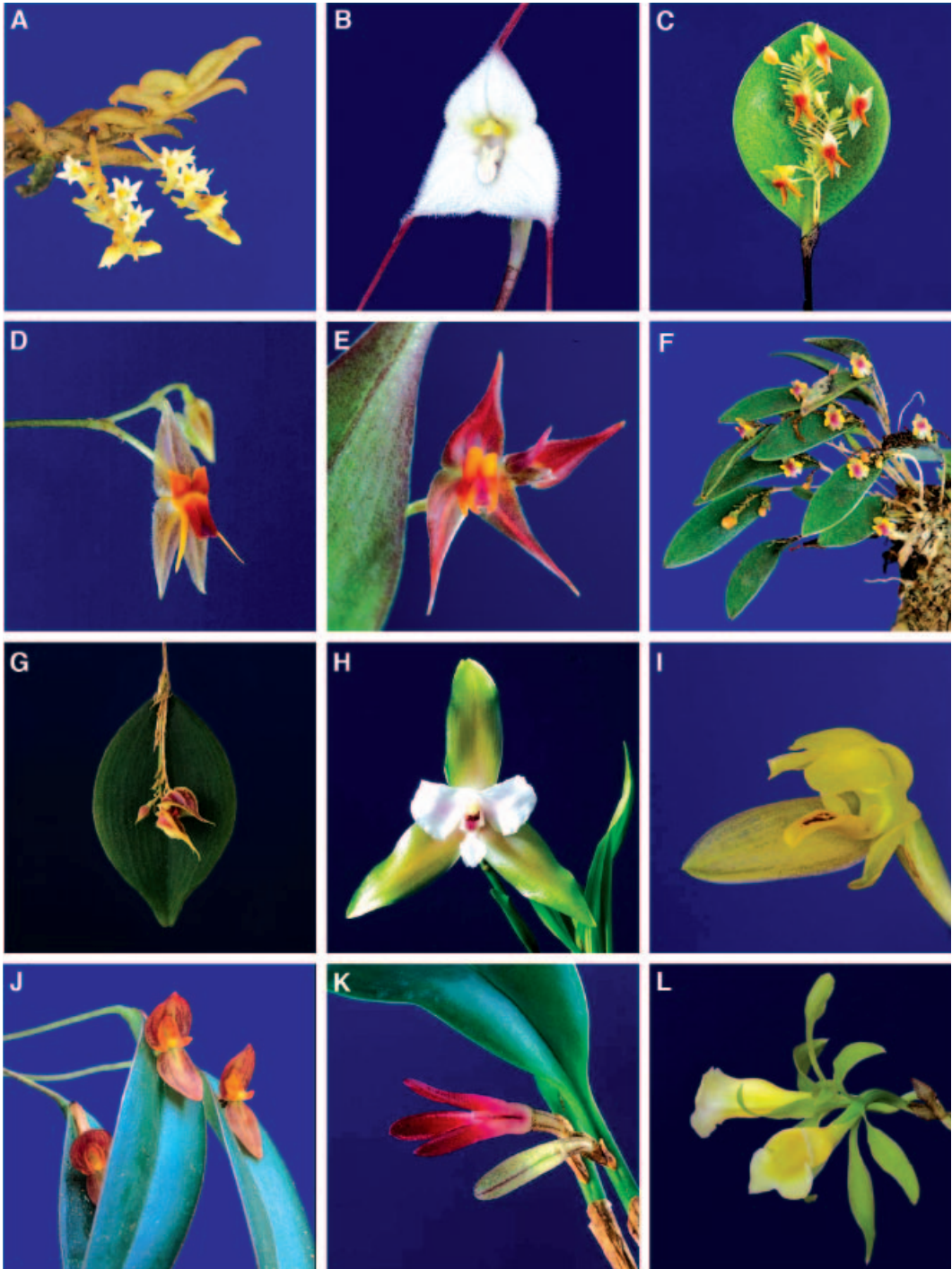


FIGURE 2. Photographs of: A. *Campylocentrum tenellum*; B. *Dracula maduroi*; C. *Lepanthes droseroides*; D. *Lepanthes gratiosa*; E. *Lepanthes machogaffensis*; F. *Lepanthes mariposa*; G. *Lepanthes pelvis*; H. *Lycaste bruncana*; I. *Mormolyca fumea*; J. *Pleurothallis bogarinii*; K. *Restrepiella lueri*; L. *Trichopilia primulina*. Photographs: A-C, F, I, K, L by Diego Bogarín; D, E, G by Franco Pupulin.

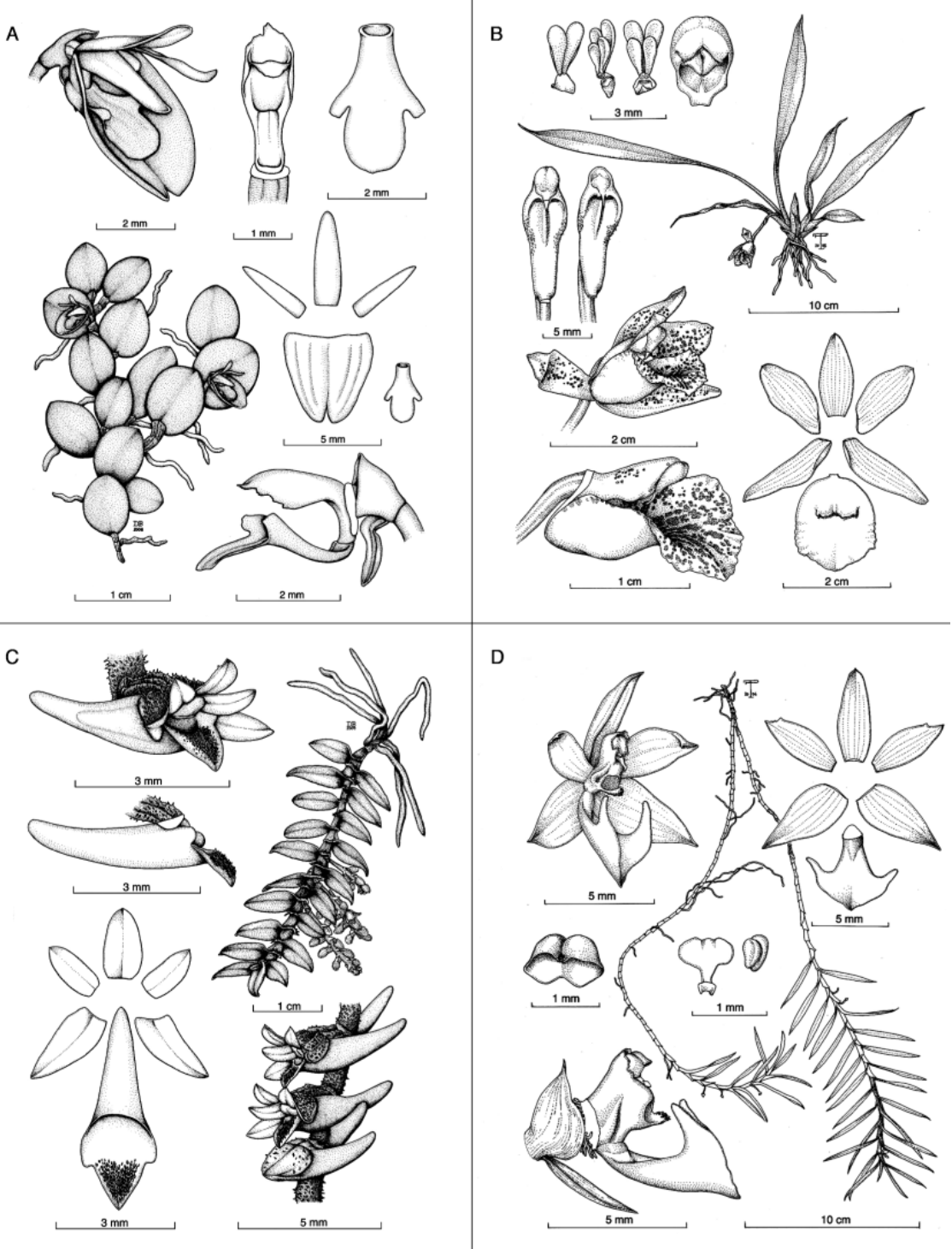


FIGURE 3. Drawings of: A. *Barbosella orbicularis*; B. \times *Bensteinia ramonensis*; C. *Campylocentrum tenellum*; D. *Dichaea gomez-lauritoi*. Drawings: A, C by Diego Bogarín; B, D by Franco Pupulin.

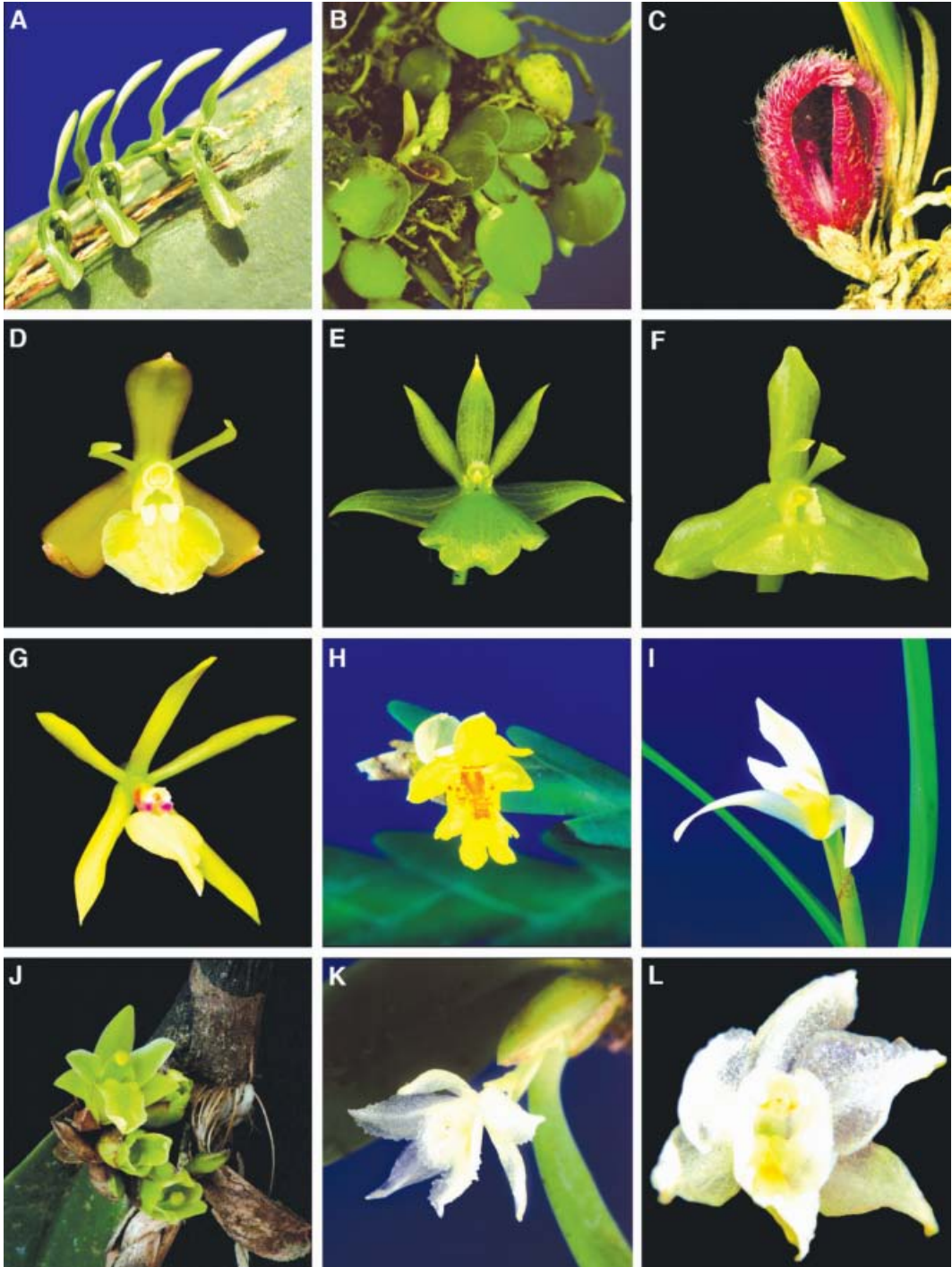


FIGURE 4. Photographs of: A. *Acianthera aberrans*; B. *Barbosella orbicularis*; C. *Brenesia lappiformis*; D. *Epidendrum maduroi*; E. *Epidendrum scharfii*; F. *Epidendrum stellidiforme*; G. *Epidendrum zunigae*; H. *Lockhartia chochoensis*; I. *Maxillaria bolivarensis*; J. *Scaphyglottis robusta*; K. *Warmingia zamorana* (Costa Rica); L. *Warmingia zamorana* (Ecuador); Photographs A, H, K by Franco Pupulin; B, I, J by Diego Bogarín; C-F courtesy of LBG; L courtesy of CIOA.

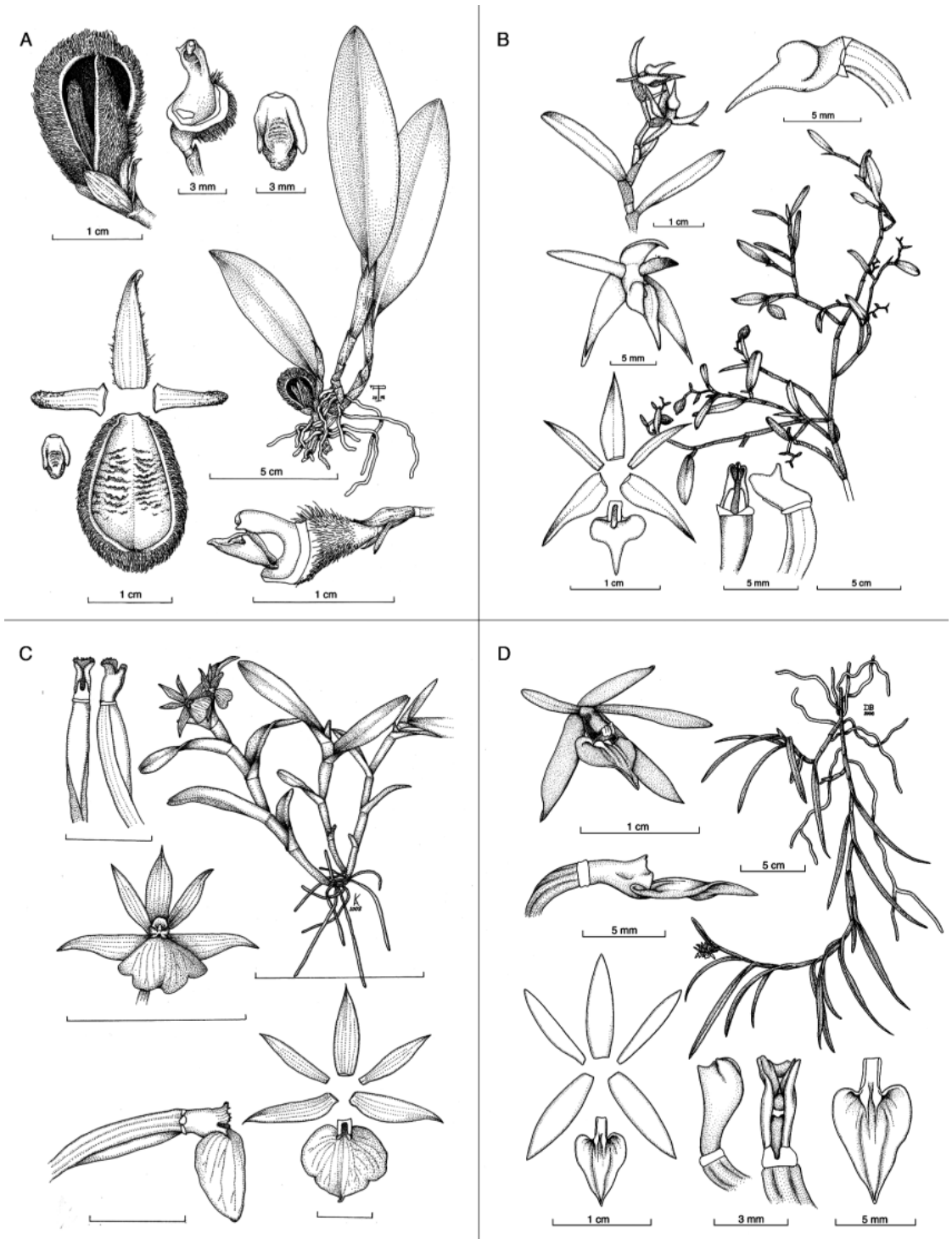


FIGURE 5. Drawings of: A. *Echinosepala lappiformis*; B. *Epidendrum orthodontum*; C. *Epidendrum stellidiforme*; D. *Epidendrum zunigae*. Drawings: A by Franco Pupulin, B, C by Adam Karremans, D by Diego Bogarín.

Subtribe Maxillariinae: The genus *Lycaste* underwent several changes and additions after studies by Bogarín (2007) and Oakeley (2008). A new species restricted to the Pacific watershed of the Talamanca range was described as *Lycaste bruncana* Bogarín (Fig. 2h). It is closely allied to *L. tricolor* Rchb.f. but differs mainly in the twisted column and the shape of the callus and pollinarium. Geographic isolation and molecular evidence also support the distinction between these two species. *Lycaste bruncana* is conspecific with the later-described *Lycaste crystallina* Wubben ex Oakeley. *Lycaste brevispatha* (Klotzsch) Lindl. & Paxton is regarded as a synonym of *L. candida* Lindl., and, according to Oakeley (2008), *L. angelae* Oakeley should replace *L. brevispatha*. However, the application of the names *Lycaste brevispatha* and *Lycaste candida* is controversial. Lindley (1851) cited *Lycaste candida* as a *nomen nudum*. Later, in 1851, J. F. Klotzsch published *Maxillaria brevispatha*, which Lindley and Paxton transferred to *Lycaste* in 1853. Then Reichenbach recognized the distinctiveness

of *L. candida* and validated Lindley's name in 1863. Oakeley (2008) regarded *L. brevispatha* as a synonym of *L. candida* but referring to the name *L. candida* Lindl. *nom. nud.* and not to *L. candida* Lindl. ex Rchb.f. of 1863. The latter is predated by *L. brevispatha* (Klotzsch) Lindl. & Paxton. So, if the two taxa are conspecific, *L. brevispatha* has priority over *L. candida*. A careful analysis of the application of the names is needed to clarify the status of the species of this group. A natural hybrid *Lycaste* x *daniloi* Oakeley is also reported as a cross between *L. angelae* and *L. candida*. *Lycaste angelae* var. *rubra* Oakeley was proposed, but it was ostensibly typified by a photo, and therefore it should be regarded as an illegitimate name according to the *International Code of Botanical Nomenclature* (Art. 37.4) which specifies that the type must be a specimen after 1 January 2007. The nomenclature of the *Lycaste macrophylla* (Poepp. & Endl.) Lindl. complex, formerly represented in Costa Rica by three subspecies (*L. macrophylla* subsp. *desboisiana* (Cogn.) Fowlie, *L. macrophylla* subsp.

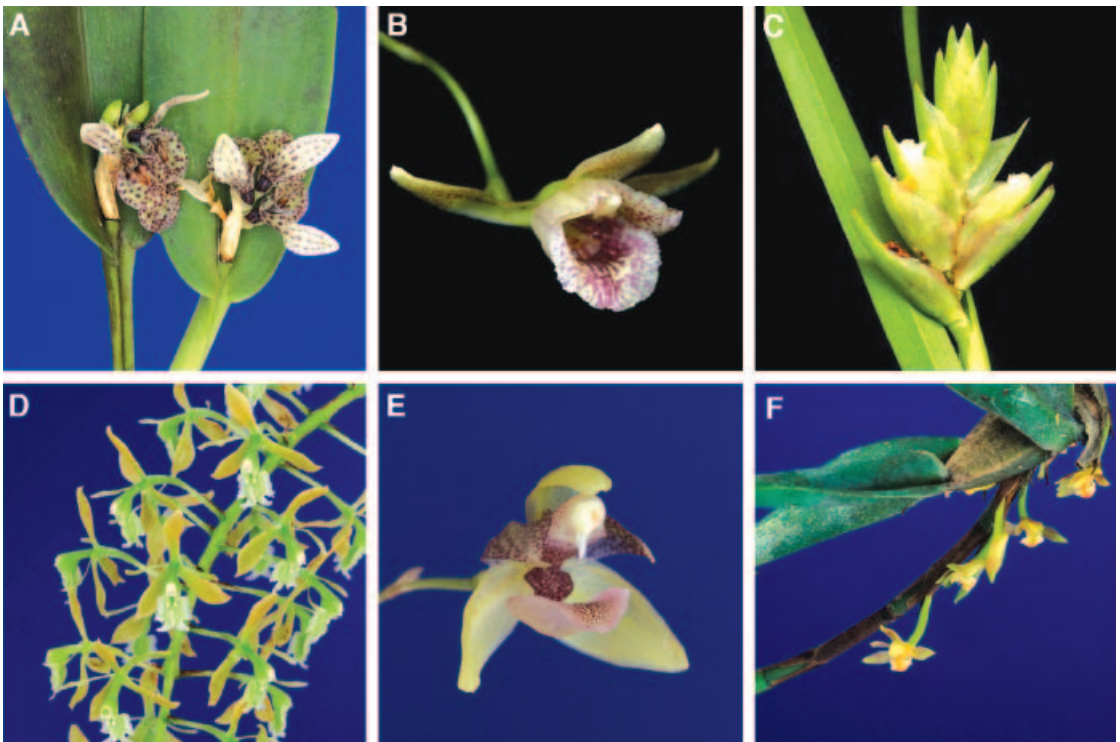


FIGURE 6. Photographs of: A. *Acianthera hamata*; B. *x Bensteinia ramonensis*; C. *Elleanthus ligularis*; D. *Epidendrum isthmioides*; E. *Kefersteinia saccata*; F. *Ornithidium pendulum*. Photographs: A, D, K, E, F by Diego Bogarín; B, courtesy of LBG.

puntarenasensis Fowlie, and *L. macrophylla* subsp. *xanthocheila* Fowlie), also changed in Oakeley (2008). He segregated the taxa within this complex, attributing to Costa Rica the following species: *L. xanthocheila* (Fowlie) Oakeley, *L. panamanensis* Fowlie ex Oakeley (first record for Costa Rica), *L. puntarenasensis* (Fowlie) Oakeley, and *L. measuresiana* (B.S.Williams) Oakeley. *Lycaste macrophylla* subsp. *desboisiana* was treated as synonym of *L. measuresiana* (Oakeley, 2008). However, a previous publication by Archila (2002) seems to have priority over Oakeley (2008), so that the correct names are *Lycaste puntarenasensis* (Fowlie) Archila, *Lycaste xanthocheila* (Fowlie) Archila, and *Lycaste panamanensis* (Fowlie) Archila.

There are new additions to various genera segregated from *Maxillaria s.l.* (Blanco *et al.*, 2007). *Maxillaria bolivarensis* C.Schweinf. described from Venezuela and distributed throughout South America has been recorded based on two collections along the Caribbean lowlands (Fig. 4I, 7b); the recent nomenclatural changes did not affect this name (Blanco 2008). *Ornithidium pendulum* (Poepp. & Endl.) Cogn. (= *Maxillaria pendula* (Poepp. & Endl.) C.Schweinf.), a widespread species ranging from Guatemala, Nicaragua, Panama, and South America, is recorded for a first time in Costa Rica (Fig. 6f, 7c). The misapplied name *Maxillaria ramosa* Ruiz & Pav. has been widely used since its clarification by Blanco *et al.* (2008). *Maxillaria appendiculoides* C.Schweinf., first described from Costa Rica, has recently been reported from Ecuador (M. Blanco, personal communication). A new species of *Mormolyca*, allied to what we call the *Maxillaria rufescens* alliance, was described by Bogarín and Pupulin (2010) (Fig. 2i). Tribe Neottieae: *Palmorchis nitida* Dressler, previously described from Panama, was documented in the Golfo Dulce region of southern Costa Rica by Bainbridge and Aguilar (2008). Subtribe Oncidiinae: A collection of *Lockhartia* similar to *L. micrantha* Rehb.f. has been reported as the Colombian *Lockhartia chocöensis* Kränzl. based on two collections from the tropical wet forest-premontane belt transition on the Caribbean watershed of the Cordillera de Tilarán range northwestern Costa Rica (Fig. 4h, 7a). Studies in *Trichopilia* revealed a new species, *T. primulina* Dressler & Bogarín, from a cultivated plant without specific locality (Dressler and Bogarín, 2009; Fig. 2l, 11b). A recent collection is the first known locality of this species. The only record of *Warmingia*

in Costa Rica, *W. margaritacea* B. Johans., is now considered a synonym of the Ecuadorian *W. zamorana* Dodson (Bogarín *et al.*, 2007; Fig. 4k, 4l, 11c, 11d).

Subtribe Pleurothallidinae: This is one of the largest groups represented in Costa Rica. After the significant contributions by Luer (1987, 2003) and studies by Pupulin (2002), Pupulin and Bogarín (2007), and Pupulin *et al.* (2007), seven new species and two new records have been registered. Three new species of *Acianthera* have been described: *A. cabiriae* Pupulin, G.A.Rojas & J.D.Zúñiga (Fig. 8c), *A. fecunda* Pupulin, G.A.Rojas & J.D.Zúñiga (Fig. 8b), both of these from the Caribbean lowlands of the Turrialba region, and *A. hamata* Pupulin & G.A.Rojas, from an unknown locality (Pupulin *et al.*, 2007; Fig. 8 d). A collection from southern Cartago corresponds to the first locality known for *A. hamata*. All the new species are endemic to Costa Rica (Fig. 6a). A new record, *A. aberrans* (Luer) Pupulin & Bogarín has been found along the Caribbean lowlands in tropical rain forest of the Sarapiquí region (Fig. 4a, 8a). This species was described from Veraguas, Panama.

The Panamanian *Barbosella orbicularis* Luer, previously cited by Pupulin (2002) on the basis of a sterile voucher, was registered from tropical wet forest along the Caribbean watershed of the Cordillera Central (Fig. 3a, 4b). A specimen of *Dracula maduroi* Luer (previously described from Bocas del Toro, Panama) was documented in a recent expedition to Volcán Cacho Negro in the Braulio Carrillo National Park in the Cordillera Central (Fig. 2b). A specimen of *Dryadella fuchsii* Luer, described from Honduras, was cited for Costa Rica by Luer (2005) based on collections by P. C. Standley and J. Valerio around the Tilarán area in the northwestern Costa Rica. The species had not been listed for Costa Rica by Luer (2003) or Ossenbach *et al.* (2007). The Nicaraguan *Echinosepala lappiformis* (A.H.Heller & L.O.Williams) Pridgeon & M.W.Chase (= *Brenesia lappiformis* (A.H.Heller & L.O.Williams)) Luer was recorded based on two collections from the Caribbean lowlands at 500 m of elevation (Fig. 4c, 5a). Another specimen was collected near Guápiles, Limón, and flowered recently in cultivation.

Studies on *Lepanthes* yielded many new records and species in last few years. Three species – *L. gratiosa* Pupulin & D.Jiménez (Fig. 1d), *L.*

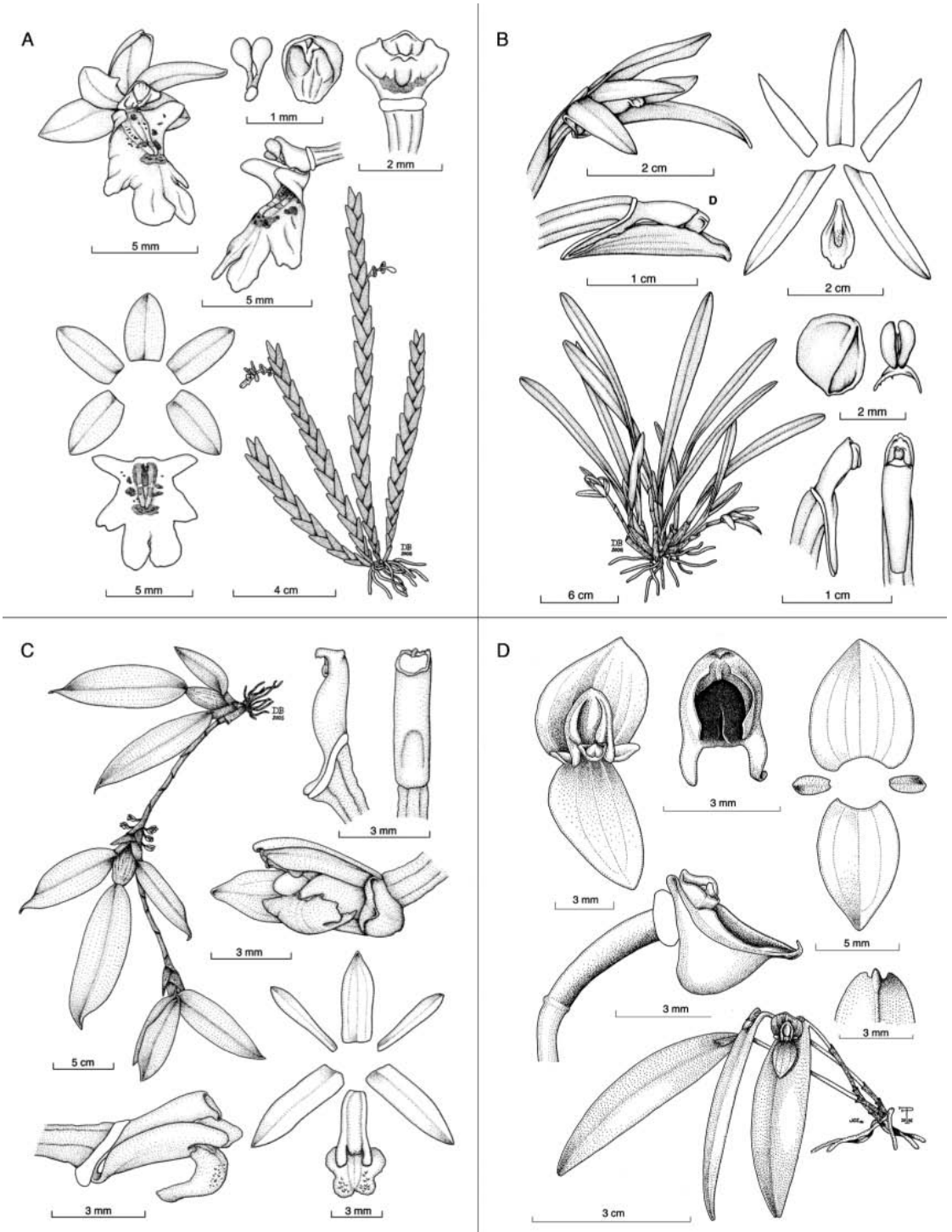


FIGURE 7. Drawings of: A. *Lockhartia chocoensis*; B. *Maxillaria bolivarensis*; C. *Ornithidium pendulum*; D. *Pleurothallis bogarinii*. Drawings: A, B, C by Diego Bogarín; D by Jose Daniel Zúñiga and Franco Pupulin.

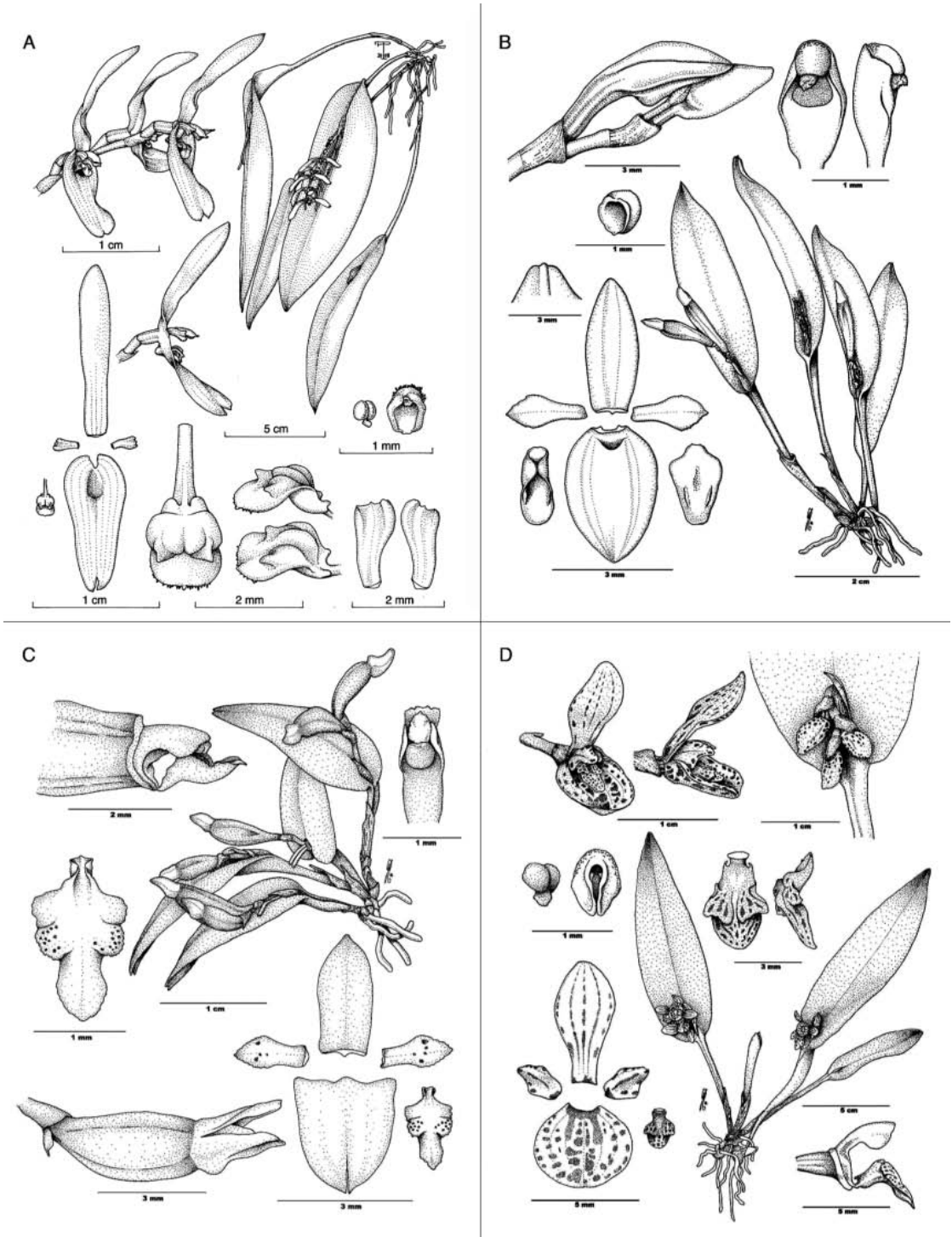


FIGURE 8. Drawings of: A. *Acianthera aberrans*; B. *Acianthera cabiriae*; C. *Acianthera fecunda*; D. *Acianthera hamata*. Drawings: A by Franco Pupulin; B, C, D by Franco Pupulin and Gustavo Rojas.

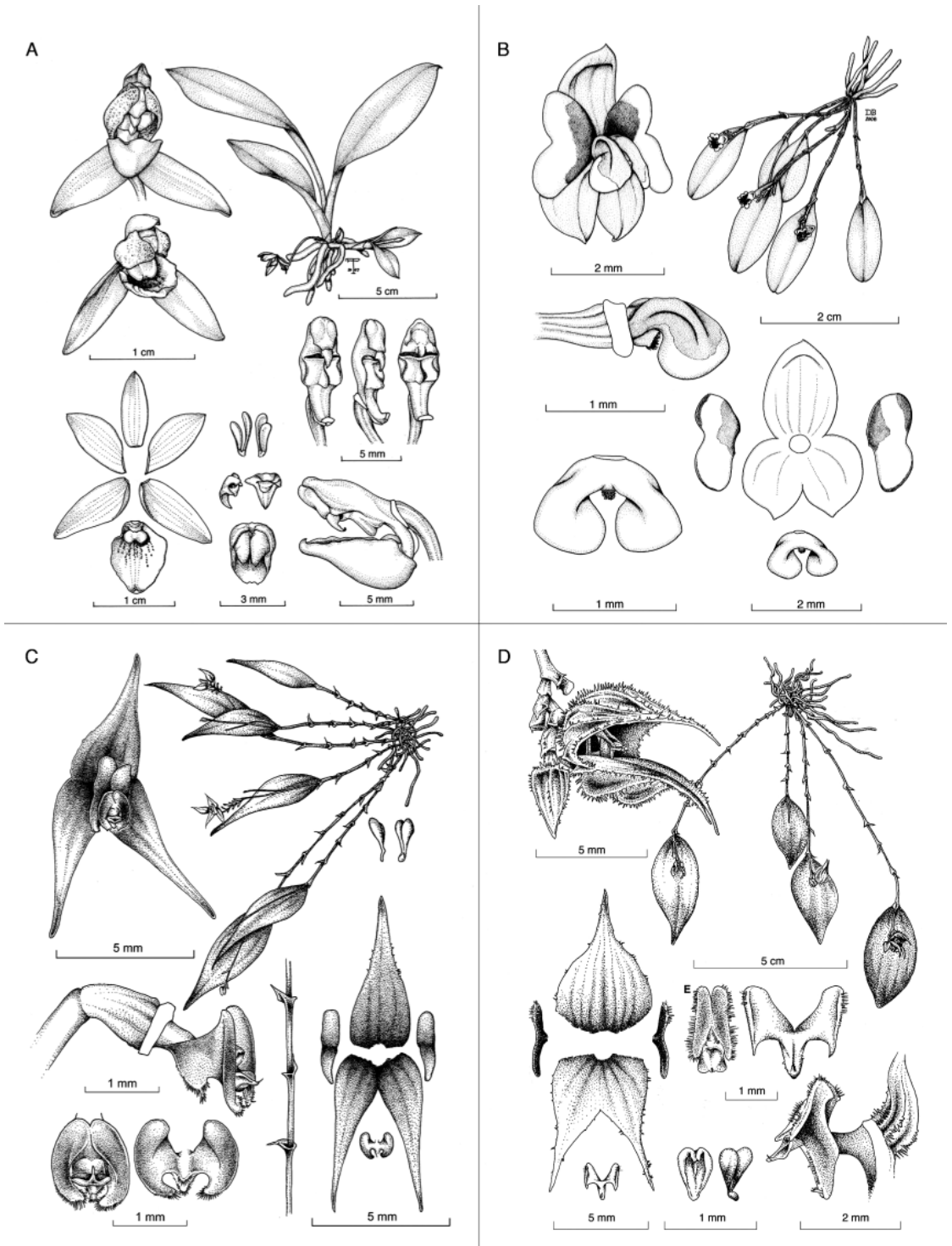


FIGURE 9. Drawings of: A. *Kefersteinia saccata*; B. *Lepanthes mariposa*; C. *Lepanthes machogaffensis*; D. *Lepanthes pelvis*. Drawings: A by Franco Pupulin; B by Diego Bogarín; C, D by Daniel Jiménez and Franco Pupulin. Pupulin.

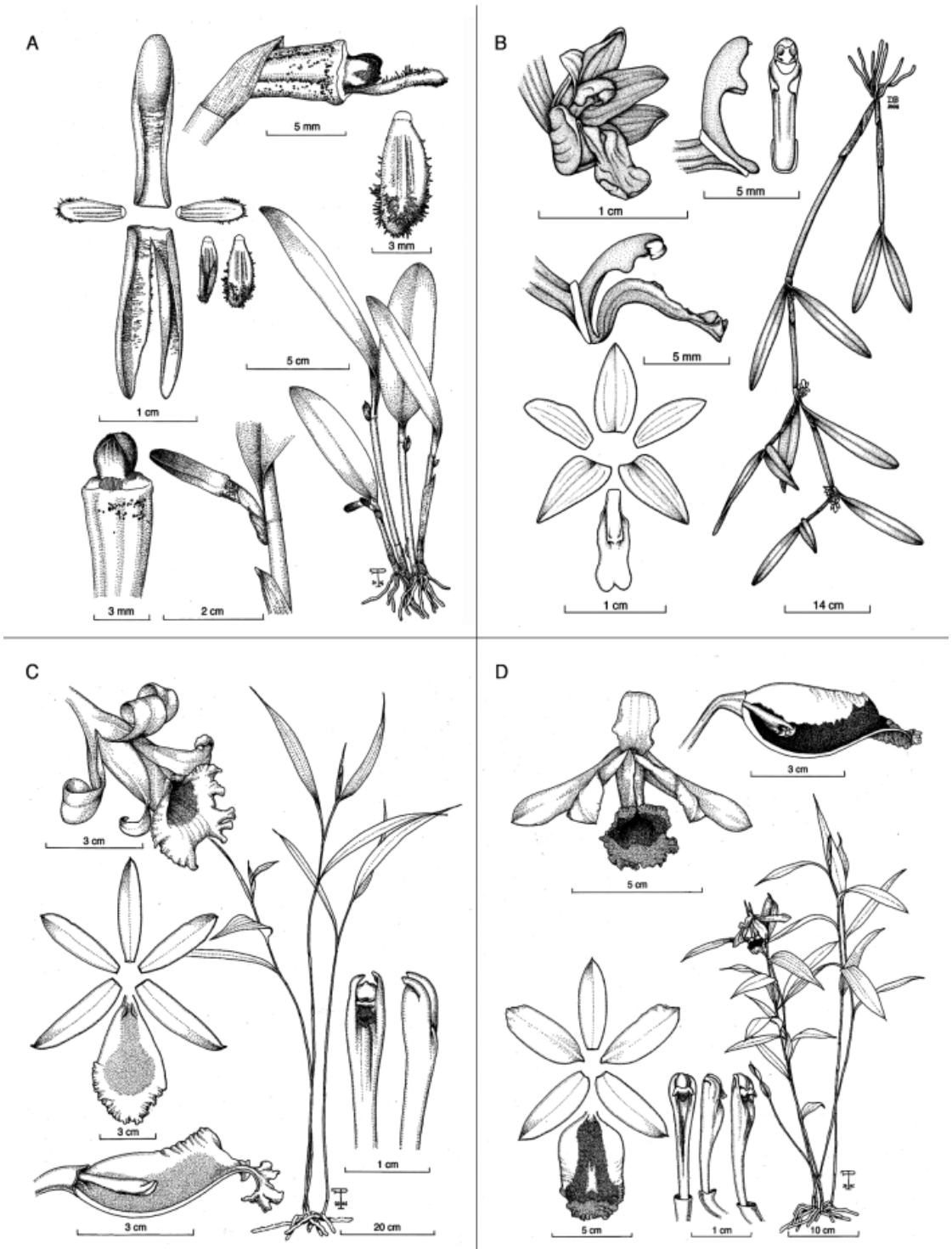


FIGURE 10. Drawings of: A. *Restrepiella lueri*; B. *Scaphyglottis robusta*; C. *Sobralia aspera*; D. *Sobralia blancoi*. Drawings: A, C, D by Franco Pupulin; B by Diego Bogarín.

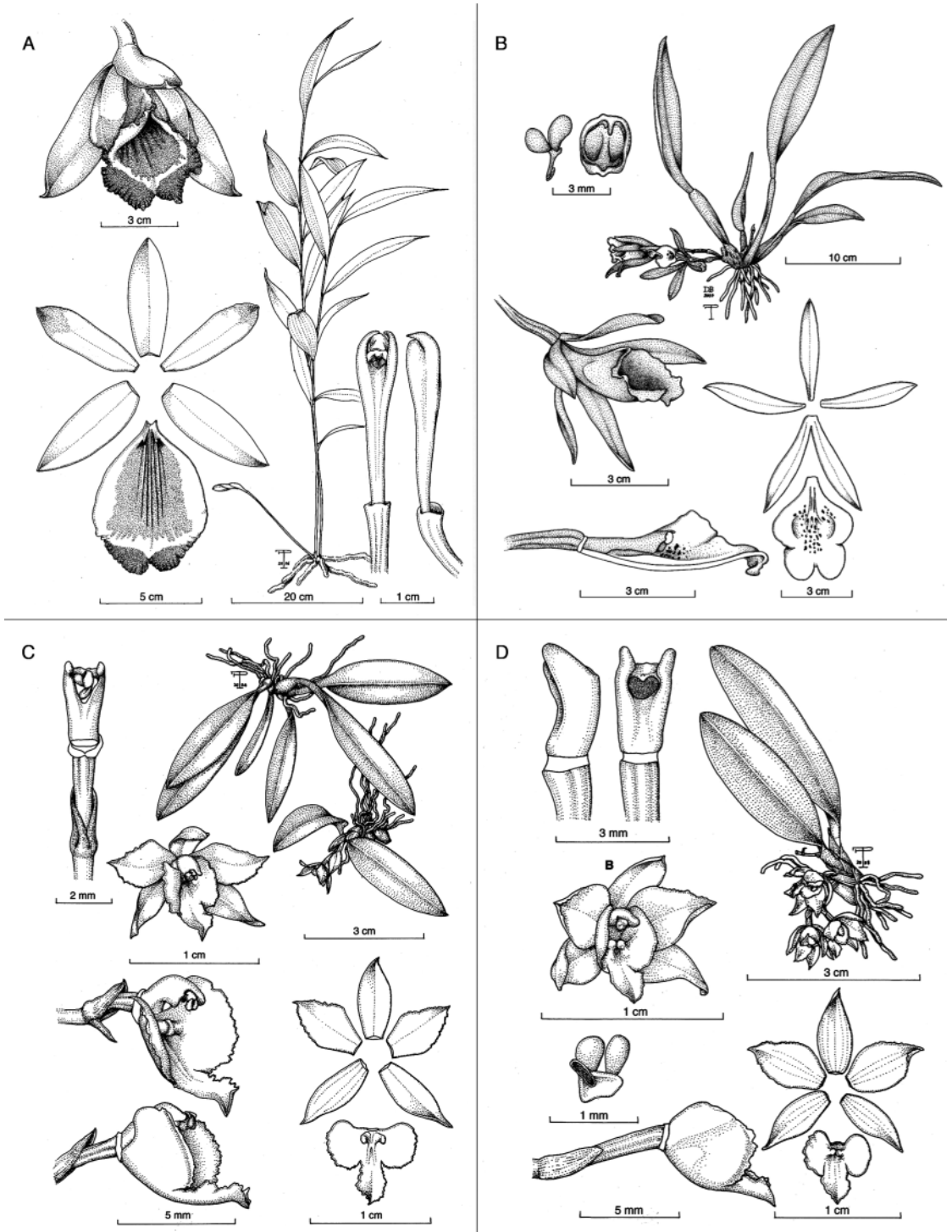


FIGURE 11. Drawings of: A. *Sobralia pendula*; B. *Trichopilia primulina*; C. *Warmingia zamorana* (Costa Rica); D. *Warmingia zamorana* (Ecuador). Drawings: A, C, D by Franco Pupulin; B by Franco Pupulin and Diego Bogarin.

machogaffensis Pupulin & D.Jiménez (Fig. 2e, 9c), and *L. pelvis* Pupulin & D.Jiménez (Fig. 2g, 9d) from the Cordillera de Talamanca — were recorded from a single path in Tapantí National Park. New collections apart from those already known have been registered for *L. pelvis* and *L. machogaffensis*. Also, *L. droseroides* Luer (Fig. 2c), *L. mariposa* Luer (Fig. 2f, 9b), and *L. pexa* Luer (of which it has not been possible to prepare a proper voucher from a cultivated plant collected in Central Volcanic range), all formerly endemic to Panama, have been collected in Costa Rica. Unpublished manuscripts will deal with more novelties in this genus (Bogarín, unpublished; Pupulin and Bogarín, unpublished).

Myoxanthus speciosus (Luer) Luer was cited by Pupulin (2002) and Luer (2003) based on a plant cultivated in Switzerland and collected in “Cordillera de Talamanca, Sierra Hills near Corazón de Jesús, alt. 2100 m” in Puntarenas Province. Luer (2003) stated that its origin is doubtful in all the collection details including the country. However, material collected near Cerro de La Muerte, along the Panamerican Highway and flowered in the living collections at the Lankester Botanical Garden confirms the presence of this species in Costa Rica. A new *Pleurothallis*, *P. bogarinii* Pupulin & J.D.Zúñiga, allied to *P. saphipetala* Luer and restricted to the Caribbean lowlands, has been registered based on two collections (Fig. 2j, 7d).

The former monospecific genus *Restrepiella* has a second species: *Restrepiella lueri* Pupulin & Bogarín (Fig. 2k, 10a). It differs from *Restrepiella ophiocephala* (Lindl.) Garay & Dunst. in the lip without basal lobes, hirsute at apex and ciliate along the margins, twice as long as the column and as long as the petals, the column without a foot and the free lateral sepals.

Two additional records added by Luer (2006) are: *Pleurothallis caudatipetala* C.Schweinf. (= *Specklinia caudatipetala* (C.Schweinf.) Luer, = *Panmorphia caudatipetala* (C.Schweinf.) Luer), previously known from Peru and Ecuador and collected in Costa Rica around Cascajal, northern San José; and *P. minutalis* Lindl. (*Panmorphia minutalis* (Lindl.) Luer, = *Specklinia minutalis* (Lindl.) Luer, = *Anathallis minutalis* (Lindl.) Pridgeon & M.W. Chase), known from Mexico and Guatemala, and based on a collection of A.R. Endrés (*Endrés s.n.*, W). A specimen of

Pleurothallis duplooyi Luer & Sayers (= *Specklinia duplooyi* (Luer & Sayers) Luer, = *Panmorphia duplooyi* (Luer & Sayers) Luer), formerly considered endemic to Belize, has been found in the Caribbean watershed of the Cordillera de Talamanca near Pejibaye in Cartago province. Plants are recognized by the shortly repent rhizome with prostrate lenticular leaves less than 8 mm long and purple flowers on a filiform peduncle. The specimen collected in Costa Rica shows an elliptic dorsal sepal rather than oblong, and basally wider lateral sepals; however, it is consistent with the drawing published by Luer (2006). The plant was sketched from a fertile specimen collected in the field, and one flower has been stored in the spirit collection at JBL. There is currently a project on the taxonomy and phylogeny of the Pleurothallidinae in Costa Rica being developed at the Lankester Botanical Garden. Without doubt many new additions will be revealed in the future.

Tribe Sobralieae: Ephemeral flowers, such as those of *Sobralia*, pose special problems for their identification, but a large living collection held at the Lankester Botanical Garden has allowed discovery of many new species. *Sobralia aspera* Dressler & Pupulin (Fig. 9c), *S. blancoi* Dressler & Pupulin (Fig. 9d), *S. pendula* Dressler & Pupulin (Fig. 11a), and *S. rareavis* Dressler were described as new, and a new record, the Panamanian *S. bouchei* Ames & C.Schweinf., was found in Turrialba on the Caribbean lowlands. Three other new species of *Sobralia* are awaiting publication (Dressler and Bogarín, unpublished). In *Elleanthus*, there is a new species, *E. ligularis* Dressler & Bogarín (Dressler and Bogarín, 2007; Fig. 6c). Plants were collected in Colombia, Costa Rica, Cuba, and Panama, but the species had remained unnamed in the absence of flowers. This species is similar to *E. graminifolius* (Barb.Rodr.) Løjtnant, but the plants are more robust with wider leaves. The blade of the lip is subquadrate rather than obtriangular, with a short base.

Subtribe Stanhopeinae: A new *Gongora*, *Gongora boracayanensis* Jenny, Dalström & W.E.Higgins, was published from the Fila Costeña on the Pacific slope as result of a clarification of the misapplied use of the name *Gongora quinquenervis* Ruiz & Pav. for a horticulturally known but scientifically undescribed *Gongora* from Costa Rica (Jenny *et al.*, 2007).

Subtribe Zygotetalinae: This subtribe has been well documented and revised in Costa Rica by Pupulin (2007). *x Bensteinia ramonensis* Pupulin, a natural bigeneric hybrid (*Benzingia reichenbachiana* (Schltr.) Dressler *x Kefersteinia excentrica* Dressler & Mora-Ret.), was recorded in the Alberto Brenes Reserve on the Cordillera de Tilarán (Fig. 3b, 6b). In the revision of *Dichaea* of Costa Rica (Pupulin, 2007), a new species, *Dichaea gomez-lauritoi* Pupulin, was described from a specimen collected in Guápiles along the Caribbean plains of Limón (Fig. 3d). Four name changes are noted: *Dichaea amparoana* Schltr. (described from Costa Rica) is recognized as different from *D. lankesteri* Ames and removed from its synonymy. *Dichaea standleyi* Ames was placed as a synonym of *D. acrolephrara* Schltr. *Dichaea schlechteri* Folsom is now considered conspecific with *D. similis* Schltr. (formerly synonymized under *D. cryptarrhena* Rchb.f. ex Kränzl.). *Dichaea ciliolata* Rolfe was reduced to a synonym of *D. hystricina* Rchb.f. based on morphological observations (Pupulin, 2005). Two novelties appeared after the revision of *Kefersteinia* (Pupulin, 2001): *Kefersteinia saccata* Pupulin from the Caribbean lowlands (Pupulin and Merino, 2008; Fig. 6e, 9a), and a new record, the Panamanian *K. alata*, collected along Yorkín river on the Costa Rican side of the border with Panama (Pupulin, personal communication).

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MATING SYSTEMS IN THE PLEUROTHALLIDINAE (ORCHIDACEAE): EVOLUTIONARY AND SYSTEMATIC IMPLICATIONS

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ABSTRACT. We developed a project addressing the determination of the reproductive system through experimental pollinations of species in the major genera representing all major lineages of Pleurothallidinae in order to determine occurrence of self-incompatibility in the subtribe, in which group it has possibly appeared for the first time, and how many times it has evolved. Additionally we surveyed the floral biology of species of *Octomeria*, a genus with morphological characters typical of bee-pollinated flowers that was previously regarded as mellitophilous. At the moment, all but one of the species studied in selected large genera of the major lineages (*Acianthera*, *Anathallis*, *Masdevallia*, *Octomeria*, *Specklinia*, and *Stelis*) are self-incompatible. The species studied may possess complete, strong or partial self-incompatibility. We found two different sites where self-incompatibility reactions occur, the stigma and the stylar channel, and both sites were not found in the same genus except for *Anathallis*. In *Anathallis*, the two groups that differ morphologically (formerly *Pleurothallis* subgen. *Specklinia* sect. *Muscosae* and sect. *Acuminatae*) exhibit different sites of reaction. Flowers of *Octomeria* species produce nectar and are pollinated by both male and female Sciaridae flies, and the populations have high genetic variability similar to that found in *Acianthera* species. Our results indicate that self-incompatibility is a generalized widespread feature of the myophilous clade of the Pleurothallidinae and possibly evolved early in the subtribe, enabling the maintenance of high levels of genetic variability in the populations of each species in spite of pollinator behavior. Moreover, although sapromyophily (pollination by female flies by deceit) is much more common in the group, myophily *s.s.* (pollination by anthophilous flies seeking nectar) apparently evolved first from the plesiomorphic mellitophilous condition. In addition to the morphological synapomorphy uniting the members of the former circumscription of the subtribe (an articulation between the ovary and pedicel), our results also point to the occurrence of two biological synapomorphies in the group — self-incompatibility and myophily. Based on these differences, we suggest the splitting of Pleurothallidinae as presently recognized into two subtribes: one composed of the large, self-incompatible, myophilous clade and the other of the small, self-compatible, ornithophilous clade.

RESUMEN. Desarrollamos un proyecto dirigido a determinar, a través de polinización experimental, la auto-incompatibilidad de los géneros representativos de los linajes más importantes de Pleurothallidinae, buscando el grupo donde posiblemente apareció por primera vez y cuántas veces ha ocurrido desde entonces. Adicionalmente estudiamos la biología floral de algunas especies de *Octomeria*, un género con características típicas de plantas con flores polinizadas por abejas, que previamente han sido conocidas como melitófilas. Hasta el momento, todas excepto una, de las especies estudiadas de los géneros seleccionados (*Acianthera*, *Anathallis*, *Masdevallia*, *Octomeria*, *Specklinia*, and *Stelis*) son auto-incompatibles, pudiendo ser fuerte o parcialmente auto-incompatibles. Se encontró dos sitios donde las reacciones de auto-incompatibilidad podrían ocurrir, siendo éstas el estigma y el canal del estilo. Los dos sitios de auto-incompatibilidad no fueron encontrados dentro de las especies del mismo género, a excepción de *Anathallis*. En este género, los dos grupos que difieren morfológicamente (anteriormente *Pleurothallis* subgen. *Specklinia* sect. *Muscosae* and sect. *Acuminatae*) presentan diferentes sitios de auto-incompatibilidad. Las flores de las especies de *Octomeria*, producen néctar y son polinizadas tanto por machos y hembras de moscas Sciaridae y sus poblaciones tienen alta variabilidad genética, similar a la encontrada en las especies de *Acianthera*. Nuestros resultados indican que la auto-incompatibilidad es una característica generalizada y ampliamente distribuida del clado miófilo de Pleurothallidinae y que posiblemente evolucionó tempranamente

en la subtribu, permitiendo el mantenimiento de un alto nivel de variabilidad genética en las poblaciones de cada especie a pesar del comportamiento de su polinizador. Más aún, a pesar que la sapromiofilia es mucho más común en el grupo, la miofilia s.s. (polinización por moscas antófilas que buscan néctar) aparentemente se desarrolló a partir de una condición melitófila plesimórfica. Adicionalmente a la morfología sinapomórfica que une a los miembros de la subtribu (una articulación entre el ovario y el pedicelo) nuestros resultados apuntan a la presencia de dos sinapomorfias biológicas dentro del grupo, la auto-incompatibilidad y la miofilia. Basados en estas diferencias, sugerimos la división de Pleurothallidinae, como está actualmente reconocida, en dos subtribus: una compuesta por el gran clado de especies auto-incompatibles y miófilas; la segunda, compuesta por el clado de especies auto-compatibles y ornitófilas.

KEY WORDS: Orchidaceae, Pleurothallidinae, reproductive system, pollination, self-incompatibility

The high variation in floral morphology displayed by orchids is mostly attributed to the striking adaptations of these plants to attract pollinators, which range from insects to birds. The morphological adaptations found in Orchidaceae favoring cross-pollination and often highly specific plant-pollinator relationships are considered largely responsible for the great numbers of species in the family (Dodson, 1962). Most orchid species are self-compatible, but self-fertilization is avoided usually by preventing self-pollination. In this family, self-pollination is generally avoided by pre-pollination barriers, which may be morphological, mechanical or ethological (van der Pijl & Dodson, 1966; Dressler, 1981; Borba & Semir, 1999; Singer & Cocucci, 1999). Apparently this mating system helps guarantee the maintenance of moderate to high levels of genetic variability within the populations. However, breeding systems alternative to the more common allogamy by pre-pollination barriers are found scattered throughout the family, such as agamospermy, self-pollination, and self-incompatibility (Tremblay *et al.*, 2005). In contrast to agamospermy and autonomous self-pollination, which are more common in Orchidoideae, self-incompatibility is more frequent in Epidendroideae, mainly in Cymbidieae, Vandeeae, and Dendrobieae (Catling & Catling, 1981; Catling, 1982; Tremblay *et al.*, 2005). Genetic barriers (i.e., self-incompatibility) have been found to be responsible for low levels of inbreeding in some species of orchids (Agnew, 1986; Johansen, 1990; Christensen, 1992; Dressler, 1993; Pedersen, 1995). Usually they are associated with species pollinated by insects that remain for a long time in flowers, a behavior that may promote autogamy or geitonogamy (Christensen, 1992; Pedersen, 1995, Borba & Semir, 1998, 1999, 2001; Singer & Cocucci, 1999; Borba *et al.*, 2001a).

The main pollinators of Orchidaceae are members of Hymenoptera, although a wide variety of other pollinator groups is known (van der Pijl and Dodson, 1966; Proctor *et al.*, 1996). Most researchers in floral biology of orchids have devoted time mainly to exploring more complex mechanisms of pollination, such as relationships with male euglossine bees, pseudocopulation mechanisms, and more species-specific relationships. Relatively little attention has been given to the reproductive biology of orchid species pollinated by flies, probably because dipterans have been considered promiscuous and inefficient pollinators (Christensen, 1994; Proctor *et al.*, 1996). However, more recently some studies have demonstrated high specificity between some orchid species and flies, mainly in the Pantropical *Bulbophyllum* (Dendrobieae) and the Neotropical Pleurothallidinae (Epidendreeae; Borba & Semir, 1998, 2001; Tan *et al.*, 2002, 2006). These two unrelated taxa are the main groups of myophilous orchids (Christensen, 1994). Myophily also occurs in many other unrelated lineages within the family, indicating that pollination by dipterans is a characteristic that has appeared several times in orchid phylogeny (van der Pijl & Dodson, 1966; Dressler, 1993; Christensen, 1994).

Pleurothallidinae comprise about 4100 species in 37 genera, encompassing 20% of the species of the family, and is the largest orchid group pollinated by Diptera. The species are characterized morphologically by the absence of pseudobulbs and the presence of an articulation between the ovary and the pedicel. An exception is seen in the clade composed by three small genera from Central America (*Dilomilis*, *Neocogniauxia*, and *Tomzanonia*), currently included in the subtribu (Pridgeon & Chase, 2001; Pridgeon *et al.*, 2001, 2005; (Fig. 1). In recent molecular phylogenetic

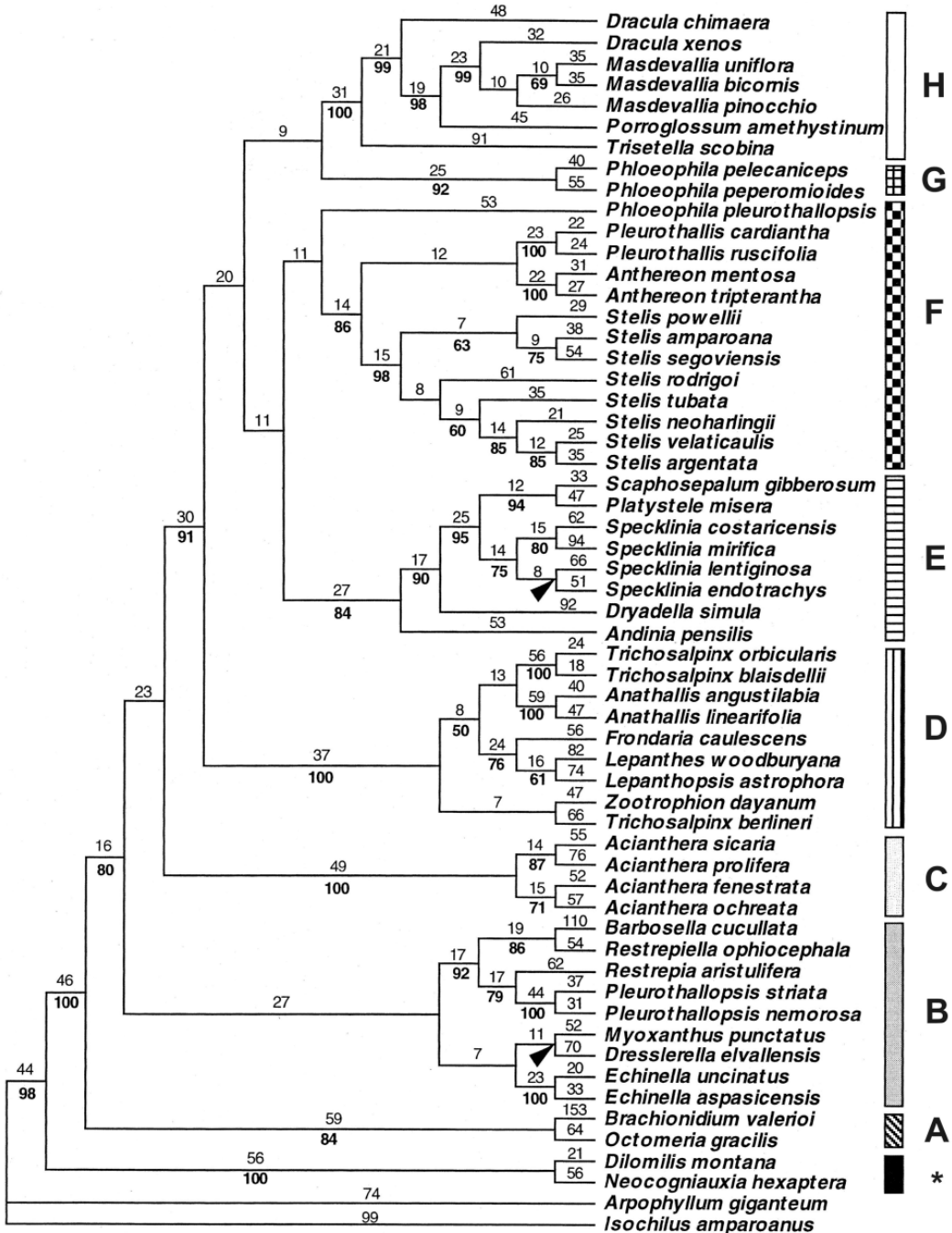


FIGURE 1. Phylogeny of the Pleurothallidinae. The single most-parsimonious, successively weighted, gap-coded *matK/trnL*/ITS DNA data set of Pridgeon *et al.* (2001) but with revised epithets. Number above branches are Fitch lengths, and those below branches are equally weighted bootstrap percentages >50%. Letters indicate the main clades according to Pridgeon *et al.* (2001); * indicates the clade included in the recent circumscription of the subtribe that previously belonged to Laeliinae. Outgroups are represented by *Arpophyllum giganteum* and *Isochilus amparoanus*. Reproduced from Pridgeon & Chase (2001) with permission.

analyses, in spite of being a monophyletic and easily recognizable group, its original circumscription was enlarged to include that small clade (a total of only eight species) of bird-pollinated, self-compatible species that previously were placed in Laeliinae (Pridgeon *et al.*, 2001, 2005). In the combined analysis (*matK* and *trnL-F* cpDNA and ITS nrDNA) of this recent phylogenetic analysis, the clade consisting of *Octomeria* and *Brachionidium* is the sister group to the rest of the members in the older delimitation of the subtribe (*sensu* Luer, 1986; Dressler, 1993). In *trnL-F* and ITS separate analyses, *Octomeria* alone is the sister group of the remaining Pleurothallidinae (*sensu* Luer, 1986). The whole larger subtribe is sister to Laeliinae. Laeliinae are primarily self-compatible and mellitophilous, but show a radiation to several bird- and lepidoptera-pollinated groups and a few self-incompatible species (Borba and Braga, 2003; van den Berg *et al.*, 2005, 2009).

Flowers of Pleurothallidinae species have a morphology, odor, and coloration typical of plants pollinated by Diptera, such as a return to radiality and presence of mobile appendages, unpleasant smell, and dull purplish and yellow colors with spots and lines (van der Pijl & Dodson, 1966; Fig. 2). Because almost all species of Pleurothallidinae are pollinated by species of Diptera, myophily is considered by some authors to be a plesiomorphic syndrome in the subtribe (van der Pijl & Dodson, 1966). However, the species of at least two of the three genera of the clade recently included in the subtribe are apparently pollinated by hummingbirds (Ackerman, 1995; Pridgeon *et al.*, 2005). Another important exception is the genus *Octomeria*, which is represented by approximately 150 species with a Neotropical distribution, concentrated mainly in northern South America and southeastern Brazil. Floral morphology is highly consistent in the genus, and it is typical in morphology, odor, and coloration typical of plants pollinated by bees (Fig. 2F-I) (van der Pijl & Dodson, 1966; Fenster *et al.*, 2004). These facts led us to question when myophily arose in the subtribe before becoming a widely occurring characteristic in the group.

Mating systems have been investigated in representatives of only three genera of Pleurothallidinae: one species of *Stelis* (Christensen, 1992), three of *Lepanthes* (Tremblay *et al.*, 2005), and

five of *Acianthera* (Borba *et al.*, 2001a; previously included in *Pleurothallis* subgen. *Acianthera*; Pridgeon and Chase, 2001; Borba, 2003), which are all self-incompatible (Tables 1, 2). All five species of *Acianthera* studied by Borba *et al.* (2001a) had reaction-site and pollen-tube morphology similar to those observed in species with homomorphic gametophytic self-incompatibility (de Nettancourt, 1977; Dafni & Calder, 1987; Murfett *et al.*, 1996; Richards, 1996). They found some features that may indicate a distinct incompatibility system, but only diallelic crossing and embryology studies can define which incompatibility system (gametophytic or sporophytic) is shown by those species (Richards, 1996; Lipow & Wyatt, 2000). *Dilomilis*, however, is self-compatible (Ackerman, 1995). This is a plesiomorphic characteristic in Laeliinae (Borba & Braga, 2003); however, self-incompatibility has evolved in some species of this group, e.g., some *Epidendrum* species (Adams & Goss, 1976; Pansarin, 2003). The distribution of self-incompatibility in remaining Epidendreae indicates that it is also a derived character in Pleurothallidinae.

Mating system and pollinator behavior are key factors determining the genetic variability of a population. The behavior of flies on flowers favors self-pollination, as they make long visits to numerous flowers on the same inflorescence or individual (Borba & Semir, 1998, 2001). Thus, because flies exhibit a behavior that enables self-pollination and orchids are typically self-compatible, we could expect that populations of myophilous orchids should have low genetic variability. However, several populations of the five *Acianthera* species studied by Borba *et al.* (2001b) showed high levels of genetic variability, much higher than previously reported for other Orchidaceae (e.g., Scacchi & De Angelis, 1989; Schlegel *et al.*, 1989; Scacchi *et al.*, 1990; Corrias *et al.*, 1991; Klier *et al.*, 1991; Case, 1994) and near the maximum known for plant species (Hamrick & Godt, 1990). Borba *et al.* (2001a) suggested that both self-incompatibility and inbreeding depression are responsible for the maintenance of high levels of genetic variability found in these species and that it may have arisen in these groups in response to pollinator behavior. A similar correlation has been also observed in *Bulbophyllum* species (Azevedo *et al.*, 2007).



FIGURE 2. Flowers of representative species of Pleurothallidinae studied. A. *Acianthera limae*; B. *A. saurocephala*; C. *Anathallis microphyta*; D. *A. sclerophylla*; E. *Masdevallia infracta*; F. *Octomeria campos-portoi*; G. *O. crassifolia*, with pollinator *Bradysia* sp. (Sciaridae) on the dorsal sepal; note the pollinarium attached to the insect's thorax; H. *O. grandiflora*; I. *O. wawrae*; J. *Specklinia pristeoglossa*; K. *Stelis* aff. *peliochyla*; L. *Stelis* sp.; M. *S.* aff. *hypnicola*; N. *Acianthera prolifera*; O-P. Mature fruits of experimentally self- (left) and cross-pollinated (right) flowers of *Acianthera prolifera*; note the least amount of seeds in the self-pollinated fruit.

Facing this scenario found so far in Pleurothallidinae, we hypothesize that self-incompatibility arose in the subtribe in response to a selection pressure caused by the change from a group that promotes cross-pollination (bees) and maintains moderate levels of heterozygosity to another group that enables a higher frequency of self-pollination (flies), which would lead

to the reduction of heterozygosity in populations. As a result of the evolution of self-incompatibility in the group, populations were able to maintain high levels of genetic variability. This hypothetical evolutionary scenario could be weakened if we found basal mellitophilous species (before the advent of myophily) with self-incompatibility or myophilous

TABLE 1. List of taxa examined of representative genera of the main clades of Pleurothallidinae (according to Pridgeon *et al.*, 2001; see Fig. 1). All sampled species in this work are native to Brazil. n= sample size.

Species	Indiv. (n)	Locality	Reference
<i>Acianthera</i> Scheidw. (clade C)			
<i>A. adamantinensis</i> (Brade) F.Barros	17	10, 13	Borba <i>et al.</i> , 2001b
<i>A. aveniformis</i> (Hoehne) C.N.Gonç. & Waechter	08	8	this study
<i>A. fabriarrosii</i> (Borba & Semir) F.Barros & F.Pinheiro	13	13	Borba <i>et al.</i> , 2001b
<i>A. hamosa</i> (Barb.Rodr.) Pridgeon & M.W.Chase	12	8	this study
<i>A. johannensis</i> (Barb.Rodr.) Pridgeon & M.W.Chase	146	8, 15, 22, 26, 28	Borba <i>et al.</i> , 2001b
<i>A. limae</i> (Porto & Brade) Pridgeon & M.W.Chase	45	9, 24	this study
<i>A. modestissima</i> (Rchb.f. & Warm.) Pridgeon & M.W.Chase	35	3, 5	this study
<i>A. ochreate</i> (Lindl.) Pridgeon & M.W.Chase	39	8, 20,	Borba <i>et al.</i> , 2001b
<i>A. prolifera</i> (Herb. ex Lindl.) Pridgeon & M.W.Chase	53	5, 9	this study
<i>A. saurocephala</i> (Lodd.) Pridgeon & M.W.Chase	06	8	this study
<i>A. teres</i> (Lindl.) Borba	86	4, 5, 10, 25, 27	Borba <i>et al.</i> , 2001b
<i>Anathallis</i> Barb.Rodr. (clade D)			
<i>A. heterophylla</i> Barb.Rodr.	9	8	this study
<i>A. microphyta</i> (Barb.Rodr.) C.O.Azevedo & Van den Berg	8	17	this study
<i>A. rubens</i> (Lindl.) Pridgeon & M.W.Chase	4	9	this study
<i>A. sclerophylla</i> (Lindl.) Pridgeon & M.W.Chase	8	5, 9	this study
<i>Lepanthes</i> Sw. (clade D)			
<i>L. rubripetala</i> Stimson	--	Central America	Tremblay <i>et al.</i> , 2005
<i>L. rupestris</i> Stimson	--	Central America	Tremblay <i>et al.</i> , 2005
<i>L. woodburyana</i> Stimson	--	Central America	Tremblay <i>et al.</i> , 2005
<i>Masdevallia</i> Ruiz & Pav. (clade H)			
<i>M. infracta</i> Lindl.	7	9	this study
<i>Octomeria</i> R.Br. (clade A)			
<i>O. alexandri</i> Schltr.	05	21	this study
<i>O. crassifolia</i> Lindl.	27	1, 5, 9, 14	this study
<i>O. grandiflora</i> Lindl.	26	2, 9, 18, 23	this study
<i>O. praestans</i> Barb. Rodr.	15	8	this study
<i>Specklinia</i> Lindl. (clade E)			
<i>S. pristeoglossa</i> (Rchb.f. & Warm.) Luer	15	12, 19	this study
<i>Specklinia</i> sp.	02	23	this study
<i>Stelis</i> Sw. (clade F)			
<i>S. argentata</i> Lindl.	--	Ecuador	Christensen, 1992
<i>S. aff. hypnicola</i> (Lindl.) Pridgeon & M.W.Chase	16	27	this study
<i>S. aff. petiochyla</i> Barb. Rodr.	11	9	this study
<i>Stelis</i> sp.	11	5, 9	this study

Localities: 1. Aiuruoca-MG; 2. Araponga-MG; 3. Belo Horizonte-MG; 4. Brumadinho-MG; 5. Caeté-MG; 6. Caldas-MG; 7. Camocim de São Félix-PE; 8. Carrancas-MG; 9. Catas Altas-MG; 10. Diamantina-MG; 11. Ecuador; 12. Funilândia-MG; 13. Grão Mogol-MG; 14. Itamarandiba-MG; 15. Itutinga-MG; 16. Jacobina-BA; 17. Joaquim Felício-MG; 18. Mariana-MG; 19. Matozinhos-MG; 20. Morro do Chapéu-BA; 21. Mucugê-BA; 22. Nazareno-MG; 23. Nova Friburgo-RJ; 24. Nova Lima-MG; 25. Ouro Preto-MG; 26. Santa Rita do Ibitipoca-MG; 27. Santana do Riacho-MG; 28. São João Del Rei-MG. Acronyms for Brazilian states: BA – Bahia; MG - Minas Gerais; PE - Pernambuco; RJ - Rio de Janeiro.

TABLE 2. Percentage of fruit set and seed viability in experimental self- and cross-pollinations and site of incompatibility reaction in species of representative genera of the main clades of Pleurothallidinae (according to Pridgeon *et al.*, 2001; see O).

Species	Fruit set % (n)			Seed viability % (min.-max.)			Self-incompatibility	Site of incompatibility reaction
	Self-pollin.	Intrapop. cross-pollin.	Interpop. cross-pollin.	Self-pollin.	Intrapop. cross-pollin.	Interpop. cross-pollin.		
<i>Acianthera</i> (clade C)								
<i>A. adamantinensis</i>	4.2 (24)	80.8 (26)	77.8 (18)	29	93 (76-97)	93 (78-98)	strong	stylar channel (base of the column)
<i>A. aveniformes</i>	0 (26)	23 (26)	--	--	--	--	complete	--
<i>A. fabiobarrosii</i>	5.3 (19)	78.6 (14)	--	33	92 (77-98)	--	strong	stylar channel (base of the column)
<i>A. hamosa</i>	0 (20)	4.3 (46)	--	0	90 (87-92)	--	complete	stylar channel (base of the column)
<i>A. johannensis</i>	12.4 (209)	59.0 (222)	66.0 (412)	20 (0-62)	90 (4-100)	90 (0-100)	partial	stylar channel (base of the column)
<i>A. lima</i>	6.6 (106)	37.1 (124)	30.3 (56)	16 (0-100)	97 (4-100)	95 (76-100)	strong	stylar channel (base of the column)
<i>A. modestissima</i>	3.3 (90)	5.1 (117)	13.1 (76)	20 (5-99)	95 (11-100)	96 (63-100)	partial	stylar channel (base of the column)
<i>A. ochreatea</i>	12.4 (89)	39.4 (132)	44.3 (176)	28 (0-87)	94 (46-100)	91 (0-100)	partial	stylar channel (base of the column)
<i>A. prolifera</i>	11.7 (137)	39.5 (162)	44.3 (61)	33 (0-100)	95 (0-100)	95 (63-100)	partial	stylar channel (base of the column)
<i>A. saurocephala</i>	0 (42)	81 (118)	--	0	82 (52-98)	--	complete	beginning of the stylar channel (distal part of the column)
<i>A. teres</i>	12.6 (159)	44.3 (167)	52.9 (240)	13 (0-86)	89 (42-99)	90 (54-99)	partial	stylar channel (base of the column)
<i>Anathallis</i> (clade D)								
<i>A. heterophylla</i>	0 (20)	33 (33)	--	0	98 (97-99)	--	complete	stigma (absence or low pollen germination)
<i>A. microphyta</i>	0 (20)	62 (29)	--	0	93 (81-99)	--	complete	stylar channel (base of the column)
<i>A. rubens</i>	0 (19)	50 (20)	--	0	95 (72-100)	--	complete	stigma (absence or low pollen germination)
<i>A. sclerophylla</i>	4 (96)	26.5 (49)	39.1 (23)	49 (44-53)	97 (94-99)	96(89-99)	strong	stigma (absence or low pollen germination)
<i>Lepanthes</i> (clade D)								
<i>L. rubripetala</i>	0.0 (11)	30.0 (44)	--	--	--	--	complete	--
<i>L. rupestris</i>	0.0 (25)	31.0 (78)	--	--	--	--	complete	--
<i>L. woodburyana</i>	0.0 (50)	60.9 (92)	--	--	--	--	complete	--
<i>Masdevallia</i> (clade H)								
<i>M. infracta</i>	32 (37)	48 (23-5)	--	73 (58-87)	93 (66-99)	--	half the population with complete self-incompatibility, the remaining self-compatible	stigma (absence or low pollen germination)
<i>Octomeria</i> (clade A)								
<i>O. alexandrii</i>	20 (5)	42 (12)	--	--	74 (35-96)	--	partial	--
<i>O. crassifolia</i>	0	88 (68)	82 (60)	0	91 (60-98)	94 (81-98)	complete	stigma (absence or low pollen germination)
<i>O. grandiflora</i>	3 (31)	84 (84)	97 (17)	65	91 (69-99)	--	strong	stigma (absence or low pollen germination)
<i>O. praestans</i>	0 (108)	84 (157)	--	0	77 (7-99)	--	complete	stigma (absence or low pollen germination)
<i>Specklinia</i> (clade E)								
<i>S. pristeoglossa</i>	7 (86)	31 (84)	24 (9)	28 (9-46)	91 (74-97)	91 (87-93)	strong	stigma (absence or low pollen germination)
<i>Specklinia</i> sp.	0 (20)	75 (8)	--	0	98 (96-99)	--	complete	stigma (absence or low pollen germination)
<i>Stelis</i> (clade F)								
<i>S. argentata</i>	1.9 (54)	29.3 (185)	--	--	--	--	strong	--
<i>S. aff. hypnicola</i>	6.9 (68)	43.2 (44)	--	2 (1-3)	91 (83-95)	--	strong	mainly stigma (absence or low pollen germination)
<i>S. aff. petiochyla</i>	0 (21)	31 (29)	--	0	93 (85-97)	--	complete	stigma (absence of pollen germination)
<i>Stelis</i> sp.	0 (39)	44 (32)	60 (5)	0	93 (84-99)	93	complete	stigma (absence of pollen germination)
<i>Zootrophion</i> (clade D)								
<i>Z. atropurpureum</i>	55(9)	58 (12)	--	70 (3-98)	98 (94-100)	--	self-compatible	normal development

self-compatible species with high genetic variability.

We developed a project addressing the determination of the reproductive system through experimental pollinations of species in the major genera representing all of the major lineages of Pleurothallidinae in order to determine: 1) the extent of occurrence of self-incompatibility in the subtribe; 2) in which group it has possibly appeared for the first time; and 3) how many times it has evolved in the subtribe. Additionally, we surveyed the floral biology of species of *Octomeria* in order to determine if myophily arose at the base of the clade that corresponds to the older circumscription of the subtribe or if it arose later in phylogeny.

Materials and methods

Mating systems — We sampled 22 species in eight genera belonging to seven of the main clades of Pleurothallidinae as defined by Pridgeon *et al.* (2001; Fig. 1, Table 1): *Acianthera* (six species), *Anathallis* (four spp.), *Masdevallia* (one), *Myoxanthus* (one), *Octomeria* (four), *Specklinia* (two), *Stelis* (three), and *Zootrophion* (one). It was not possible to sample clade G, because we could not obtain individuals of *Phloeophila* species, the only genus of this lineage. Studies with the *Myoxanthus* species sampled (clade B) are still in progress and are not presented here. Thus,

we present the results of six of the eight main clades of Pleurothallidinae. Vouchers were deposited in the herbarium BHCN (Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais).

The populations studied were located in areas of *campo rupestre* vegetation and forests in southeastern (Minas Gerais and Rio de Janeiro states) and northeastern (Bahia state) Brazil, with the individuals occurring as epiphytes in semideciduous, gallery, and Atlantic rain forests or as rupicolous on rock outcrops. Experimental pollinations were performed on cultivated plants. Individuals were collected for acclimatization and maintained for a minimum of six months in a greenhouse at the Universidade Federal de Minas Gerais. Three types of pollination experiments were performed, depending on the availability of flowers in each population. All populations were subjected to self-pollination and intrapopulation cross-pollination experiments with a tentative number of at least 20 pollinations per treatment. Interspecific crosses were performed in some species with a multipopulation sampling. We tried to use about 15 to 20 individuals per population and at least two populations per species. Sometimes the numbers were lower, but in some cases they were as high as 160 pollinations per treatment, 30 individuals per population, and six populations per species. Additionally, some marked emasculated or not emasculated flowers were followed in order to verify the possible occurrence of diplosporic agamospermy or spontaneous self-pollination. All flowers used in the pollination experiments were checked on a daily basis to verify the formation and development of fruits.

Additional self- and cross-pollinations were performed and subsequently harvested at regular intervals after pollination (ranging from two to 15 days, depending on the flower longevity of the species) in order to observe pollen-tube growth. These flowers (and senescent flowers and mature fruits of the earlier described treatments) were fixed in 50% FAA and subsequently treated with a solution of 10N NaOH at 60 C for approximately 25 min. (or 50 min. in the case of fruits). The material was then washed in distilled water and stained with Aniline Blue for examination by epifluorescence microscopy (modified from Martin, 1959).

The fruits were collected at the start of their dehiscence and fixed in 50% FAA. Approximately

300 seeds from each fruit were examined by optical microscopy and classified as viable or non-viable on a morphological basis only, according to the morphology and relative size of the embryo: seeds with well-developed embryos were considered viable, and seeds with no embryo or a rudimentary embryo were considered inviable (Fig. 3A; Borba *et al.*, 2001a).

Floral biology of Octomeria species—Field observations were made in populations of *O. crassifolia* and *O. grandiflora* in the Serra do Caraça Mountains (municipality of Catas Altas, Minas Gerais state, 20°5'36"S, 43°28'29"W) to determine their floral visitors. Observations of *O. grandiflora* were made on January and February 2007 from 07:00 to 18:00, totaling 88 hours of observations. Observations of floral visitor activities in *O. crassifolia* were made on January 2007, between 06:00 and 12:00, totaling 63 hours of observation. Floral visitors were captured and sent to specialists for identification. Here we present only a summary description of the pollination of the species with the identity of pollinators, because a detailed description of the reproductive biology of the species is being published elsewhere (Barbosa *et al.*, 2009).

Results

Fruit set and seed viability in experimental pollinations — Flowers that did not produce fruits abscised within 5-10 days, except for *Acianthera prolifera* and *Anathallis microphyta*, flowers of which abscised after 12-14 and 21 days, respectively. Fruits terminated development in approximately 60-90 days (*Anathallis heterophylla*, and *Acianthera*, *Octomeria*, *Specklinia*, *Stelis* species), 110-130 days (other *Anathallis* species) or 150 days (*Anathallis sclerophylla* and *Masdevallia infracta*). No emasculated or unpollinated flower of any species developed fruits.

Fruit set in experimental intra-population cross-pollinations was variable among species, ranging from 5 to 88% (Table 2). Fruit set in inter-population cross-pollinations was similar to that found in intra-population crossings, the latter being usually slightly lower or more rarely higher (e.g., *Acianthera limae*). Except for one species, fruit set in self-pollinations was always lower than in cross-pollination experiments, ranging from 0 to 55%, but rarely above 20% (only

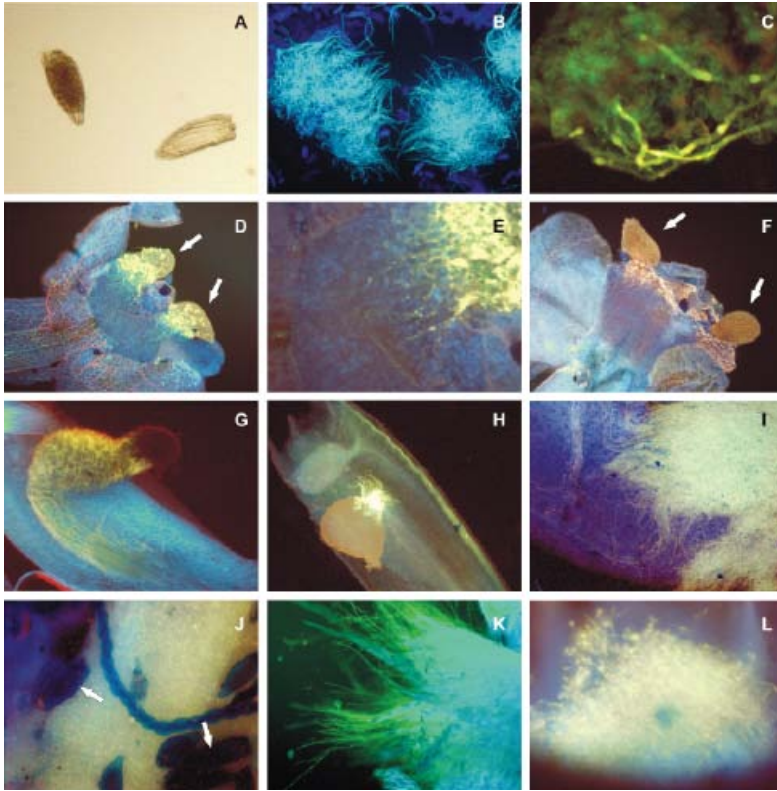


FIGURE 3. A. Normal (left; considered viable) and embryo-lacking (right; considered inviable) seeds of *Anathallis rubens* (see Materials and Methods for explanation); B-L. Pollen germination and tube growth in fluorescence microscopy. B-C. Cross- (B) and self-pollinated (C) flowers of *Octomeria crassifolia*, after three and six days, respectively. Note several pollen tubes with normal development in (B) and the rare pollen tubes in (C), the latter with irregular growth and irregular deposition of callose on the pollen tube walls; D-F. Cross- (D, E) and self-pollinated (F) flowers of *Stelis* aff. *peliochyla*, after four and six days, respectively. Note the pollinaria with germinated (D) and ungerminated (F) pollen grains (arrows); in (E) detail of pollen tubes of (D); G-H. Cross- (G) and self-pollinated (H) flowers of *Specklinia pristeoglossa*, after six and four days, respectively; note the few pollen tubes formed in (H); I. Self-pollinated flower of a self-compatible individual of *Masdevallia infracta*, with normal pollen tubes reaching the base of the column; J. Pollen tubes in a mature fruit from a cross-pollinated flower of *Masdevallia infracta*; note the well-developed mature seeds (arrows); K-L. Cross- (K) and self-pollinated (L) flowers of *Acianthera saurocephala*, after six and eight days, respectively. Note several pollen tubes with normal development in (K) and the pollen tubes in (L) with irregular growth and high deposition of callose.

two species). Ten species did not set any fruits in self-pollinations, and fruit set in this treatment was lower than 10% in other six species. Fruit size and time of development in cross-pollination fruits were usually higher than in self-pollinated fruits. The latter also had fewer seeds, and sometimes they failed to open, mainly in *Acianthera* species (Fig. 2N-P). However, for *Zootrophion atropurpureum*, fruit set in both self- and cross-pollinations was nearly 55%. In this species, they were also similar in size, time of development, and seed content in both treatments.

Although *Masdevallia infracta* had a high average fruit set in self-pollination, all fruits were developed in only four of the seven individuals sampled. These individuals had fruiting ranging from 17% (n=6) to 100% (n=1, and 2); the individual that had the largest number of self-pollinated fruits (n=9) had 67%. The other three individuals did not set any fruits with self-pollination (n=2, 2, and 9), but they did in cross-pollination (one of them with 100% fruit set in four cross-pollinations), indicating that they are not sterile. Bidirectional crossings between one pair of self-

compatible and self-incompatible individuals ($n=12$) set fruit only when the self-incompatible individual was the pollen donor ($n=4$). Another pair of similar individuals had 100% fruit set, independently of direction of crossing. Average seed viability was always high in cross-pollinated fruits, usually greater than 80% (except for *Octomeria alexandri*, 74%, and *O. praestans*, 77%; Table 2). However, seed viability in some individual fruits was possibly as low as 7% in this treatment. Conversely, average seed viability in self-pollinated fruits was usually never greater than 70% (except for *Masdevallia infracta*, 73%), and always lower than in cross-pollinated capsules (including *Z. atropurpureum*). Again, seed viability in some individual fruits in this treatment was as high as 100% but more frequently lower than 20%. We found no evidence for polyembryony in any capsule examined of any species.

Pollen germination and pollen-tube growth—In experimental cross-pollination, germination of pollen grains began at the end of the first day up to the third day after pollination, depending on the species. In this pollination treatment, practically all pollen grains germinated, and the pollen tubes demonstrated uniform growth, with callose plugs deposited regularly along their lengths (Fig. 3B, D, E, G, K). These tubes grew down through the style canal and penetrated the ovary on about the fifth to eighth day after pollination, except for *Anathallis microphyta* and *Acianthera* species, for which it occurred on the 11th day. Penetration of the ovules occurred from 20 days after pollination. In self-pollinated flowers that developed fruits, pollen-tube growth occurred in a manner similar to that observed in cases of cross-pollination.

We found two general patterns for pollen and pollen-tube behavior in self-pollinated flowers that did not develop fruits (Table 2). In *Masdevallia infracta* and all *Octomeria*, *Stelis*, *Specklinia*, and *Anathallis* (except *A. microphyta*) species, pollen grains in the majority of self-pollinated flowers did not germinate (Fig. 3C, F). However, in some flowers, the few pollen grains that did initiate germination demonstrated irregular growth and acquired a twisted appearance when they reached the initial portion of the style with irregular deposition of callose on the pollen tube walls and at the extremity of the pollen tube itself (Fig. 3H). These tubes rarely reached the base of the column and never

penetrated the ovary. In all *Acianthera* species and *Anathallis microphyta*, pollen-tube growth occurred in a way similar to that observed in cross-pollination until approximately seven days, when the tubes had penetrated halfway through the column. However, from that point onward, pollen tubes began to take on an abnormal aspect, with irregular trajectory, variation in diameter, and excessive deposition of callose. At almost 15 days, these flowers had pollen tubes with abnormal characteristics that had reached the base of the column but never penetrated the ovary. *Acianthera saurocephala* showed a slight variation of this pattern, the pollen tubes assuming abnormal appearance as above in the beginning of the styler channel (distal part of the column; Fig. 3L), but pollen tubes never reached the base of the column. In individuals of *Masdevallia infracta* that set fruit in self-pollinations, pollen tubes had normal development, similar to that found in cross-pollinated flowers (Fig. 3I, J).

Pollen tubes in fruits with a high percentage of normal seeds developed normally (Fig. 3J). However, fruits with a high percentage of empty seeds (lacking embryos) usually showed normal pollen tube growth only in the column; they became irregular when entering the ovary, filled with callose, and formed a tangled mass around the ovules. The last behavior was more frequent in fruits developed from self-pollinations, mainly in *Acianthera* species, which showed higher fruit set in this treatment than the other species.

Floral biology of Octomeria species—*Octomeria crassifolia* was pollinated exclusively by both males and females in similar proportions of four species of *Bradysia* (Diptera, Sciaridae; Fig. 1G). During 63 hours of observation, a total of 92 visits were recorded and 31 pollinarium removals and six pollinarium depositions were observed. The visits occurred more frequently early in the morning, between 06:00 and 08:00. Pollinators of *O. grandiflora* were rarely observed in flowers of *O. crassifolia*. However, pollination did not occur because of their large size. Males and females of a single species of *Pseudosciara* (Diptera, Sciaridae) pollinated flowers of *O. grandiflora*; 19 visits occurred in 88 hours of observation. Unlike those observed in *O. crassifolia*, visits occurred mainly in the afternoon between 14:00 and 16:00. In both species, the visits lasted usually less than a minute. Three visits by the

pollinators of *O. crassifolia* were observed on the flowers of *O. grandiflora*, but no pollinarium was removed due to the small size of these insects.

The pollinators of the two species demonstrated similar behavior. Generally, the insect landed on the external part of the dorsal sepal and then moved to the labellum, which shifts slightly downward. The insect continued moving toward the base of the labellum, feeding on the nectar produced on the disk. When the insect attempted to leave, the pollinarium became affixed to the dorsal region of its thorax (the scutellum) and was removed from the flower. In the population of *O. crassifolia*, 64.3% of the flowers had their pollinarium removed, and in 35.7% of the cases pollinia were successfully deposited. Deposition of pollinarium occurred in 8% of the flowers of *O. grandiflora*.

Discussion

Absence of fruit set in unpollinated or emasculated flowers and absence of polyembryony indicate that none of the studied species is capable of developing fruits by autonomous self-pollination or agamospermy; thus, a pollination is required for the formation of fruits in these species. Absence or low fruit set in self-pollination versus moderate to high fruit set in cross-pollinations indicates occurrence of complete or strong self-incompatibility in the majority of the species studied (see Table 2). Complete or strong self-incompatibility was also found in the majority of Pleurothallidinae species studied so far (Christensen, 1992; Borba *et al.*, 2001a; Tremblay *et al.*, 2005; see Table 2).

However, in some species, as observed in a previous study in *Acianthera* (Borba *et al.*, 2001a), fruit set in self-pollination was about the half that observed in cross-pollination experiments. As emphasized by Borba *et al.* (2001a), strict self-incompatibility and self-compatibility are extremes of a continuum between which there is often no clear-cut difference, and relatively few species fit exactly in these extremes. The distinction between the two conditions is arbitrary, however, and various authors have used different indices to determine whether a particular species is self-compatible or self-incompatible (e.g., Bawa 1974, 1979; Zapata and Arroyo, 1978; Sobrevilla & Arroyo, 1982; Jaimes & Ramirez, 1999). Thus, some

species in this study (e.g., *Acianthera prolifera*) could be considered self-incompatible or self-compatible depending on which method is employed. Some of these indices have combined seed viability with fruit set, which we think is improper, because seed viability is probably related to inbreeding depression. If that practice is used for these species, they would be considered clearly self-incompatible on account of the low seed viability in fruits from self-pollinated flowers. The pollen tube reaction and the homogeneous timing of the reaction in the stylar canal or absence of pollen germination lead us to suggest the occurrence of partial self-incompatibility in these species.

Conversely, *Zootrophion atropurpureum* can be clearly considered self-compatible, in spite of the reduced seed viability in fruits from self-pollination when compared to crosspollination. But *Masdevallia infracta*, which could be considered self-compatible if based simply on total fruit set, probably has a more complex mating system. Based on the individual analysis, half the population of *Masdevallia infracta* exhibit strong self-incompatibility (with the reaction site on the stigma because of little or no pollen germination), and half the population is composed of self-compatible individuals, as we could see by the bundle of normal pollen tubes in some self-pollinated flowers. Bidirectional crossings between self-compatible and self-incompatible individuals indicate that self-compatibility in these individuals may be caused by silencing of the gene responsible for the stylar self-incompatibility factor. Thus, the species, or at least this population, may have a mixed mating system, which may ensure both reproductive success and genetic variability. A similar situation was observed in *Asclepias exaltata* L., with a few self-compatible individuals occurring in a self-incompatible population (Lipow & Wyatt, 2000). This may also explain the occurrence of some fruit set in self-pollinations of those species considered as having strong or partial self-incompatibility. Unfortunately, the sample size of *Z. atropurpureum* was low, and so we cannot know whether the same occurs in this species/population or if it is in fact self-compatible. We will try to increase the number of experimental pollinations and individuals sampled to answer this question.

The species showed two different self-incompatibility reactions, one typical of gametophytic self-incompatibility (pollen tubes becoming irregular

in their trajectory, with variation in diameter and excessive deposition of callose) and the other typical of sporophytic self-incompatibility (absence of pollen grain germination; Richards, 1996). In addition to one *Anathallis* species, the reaction typical of gametophytic self-incompatibility was observed only in all *Acianthera* species. There are currently no published studies involving diallelic crosses in Orchidaceae, although they are fundamental to understanding control of self-incompatibility in the group. We need more studies to determine if this picture represents either two incompatibility systems (gametophytic and sporophytic) or two sites of the same reaction. Intermediate situations, as found in species such as *Acianthera saurocephala* and *Stelis aff. hypnicola*, indicate that the latter scenario is more plausible.

All *Anathallis* species showed strong self-incompatibility, but we found the two different sites where self-incompatibility reactions occur, the stylar channel and the stigma, which are related to the two vegetative morphological groups, formerly *Pleurothallis* subgen. *Specklinia* sect. *Muscosae* and *P.* subgen. *Acuminatia* (Luer, 1986, 1999). This division was based principally on their growth habit and the leaf-to-stem proportions of the plants. Despite differences in morphology, the new circumscription of the genus eliminated this division, and both groups were combined to form the core of the genus *Anathallis* (Pridgeon & Chase, 2001). This is the only genus in which both sites of incompatibility were found. In this same clade, there is a report of strong self-incompatibility in three *Lepanthes* species by Tremblay *et al.* (2005). In *Anathallis microphyta*, formerly included in *Pleurothallis* subgen. *Specklinia* sect. *Muscosae* and recently transferred by Luer (2006) to *Panmorphia*, germination of the pollen grains in self-pollinated flowers is normal, and the incompatibility reaction occurs when the pollen tubes reach the base of the column. On the other hand, in the three species formerly belonging to *Pleurothallis* subgen. *Acuminatia*, the pollen grains fail to germinate or pollen germination is low and the pollen tubes do not penetrate the stylar channel. We believe that in order to reach a better understanding of the distinct infrageneric relationships and the evolution of the self-incompatibility mechanisms in the genus, additional studies examining the mating systems and molecular

phylogenetic analysis are necessary, both using larger samples of species belonging to the two morphological groups.

Experiments in progress by our group indicate that self-incompatibility can also be assigned to other species of *Pleurothallidinae*, such as *Octomeria campos-portoi*, *O. diaphana*, *O. wawrae*, *Myoxanthus exasperatus*, *M. punctatus*, and additional *Acianthera* and *Specklinia* species. However, the results were not included here because of the still low sample size. Occurrence of self-incompatibility in *Myoxanthus* species is particularly important to our study, since they represent a clade not sampled in this study (see Fig. 1).

Our results indicate that self-incompatibility is a generalized, widespread characteristic of the myophilous clade of the subtribe, in spite of the occurrence of self-compatibility in *Zootrophion*, possible by reversal.

Several populations of two of these *Octomeria* species (*O. grandiflora* and *O. crassifolia*) have been scored for genetic variability (Barbosa *et al.*, unpublished), and all of them show high genetic variability, measured by using ISSR markers. The values we found are higher than the values found in other orchid species, including self-compatible *Laeliinae*, and above the average values for outcrossing plants in general. This picture is similar to that previously found in 22 populations of five *Acianthera* species by using allozymes. Self-incompatibility and inbreeding depression apparently are factors responsible for maintaining high levels of genetic variability in these populations (Borba *et al.*, 2001a, 2001b).

The phylogenetic position of *Octomeria* indicates that self-incompatibility and myophily arose at the beginning of the line that gave rise to the core group of the subtribe, corresponding to the older circumscription of this group (Luer, 1986; Dressler, 1993). As such, self-incompatibility and myophily may be considered as biological synapomorphies of this large myophilous clade. The concomitant occurrence of myophily and self-incompatibility in all of the clades studied indicates two hypothetical routes for the evolution of these two characteristics in the subtribe. In the first, the evolution of self-incompatibility would have permitted the establishment of myophily in the group. In the second, self-incompatibility would have

arisen as a result of selection pressures that arose by the change of pollinators, from an insect group with a behavior favoring cross-pollination to a group favoring autogamy, which would lead to a reduction in heterozygosity in the populations. At the present time, our results cannot distinguish which of the two characteristics was first established in the group.

Sapromyophily is apparently widely distributed and represents the predominant pollination mechanism in the subtribe (van der Pijl & Dodson, 1966; Christensen, 1994). Some characteristics of *Octomeria* flowers, such as a yellow perianth, nectar guides on the calli, nectar reward at the base of the lip, and agreeable citriny odor are found in myophilous species but are also common in mellitophilous species. Because of these and other characteristics such as a zygomorphic perianth and purple lip, *Octomeria* flowers were falsely regarded as mellitophilous (van der Pijl & Dodson, 1966). Thus, we suggest that myophily *sensu stricto* (pollination by anthophilous flies seeking for nectar) may be a plesiomorphic characteristic in the myophilous clade of the subtribe in spite of the most common occurrence of sapromyophily (pollination by female flies by deceit) and may represent a transition from rewarding melittophily to deceptive sapromyophily. Adaptive radiation from melittophily to myophily is not uncommon in orchids, and the application of pollination syndromes can be especially conflicting in these cases (e.g. Pansarin, 2008).

In the older circumscription of the subtribe, the articulation between the pedicel and the ovary was considered an important diagnostic characteristic for the group (Luer, 1986; Dressler, 1993). With the inclusion of the small clade containing the genera *Dilomilis*, *Neocogniauxia*, and *Tomzania* (which do not have this characteristic), no morphological synapomorphy is recognized for the group (Pridgeon *et al.*, 2001). For this reason, and the occurrence of self-compatibility and ornithophily in some species of this clade, we suggest that the older circumscription of the subtribe should be maintained with self-incompatibility and myophily as biological synapomorphies and the articulation as a morphological synapomorphy for Pleurothallidinae. As such, the establishment of a subtribe comprising the genera *Dilomilis*, *Neocogniauxia*, and *Tomzania* should be considered, even taking into consideration the reduced size of the group. "Taxonomic inflation"

should not be the main argument for its inclusion in the subtribe (see Pridgeon *et al.*, 2001). As these two groups are sister to each other, such taxonomic rearrangement is still supported by the results found by Pridgeon *et al.* (2001).

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A LOOK AT 'THE ORCHID BOOK' IN CELEBRATION OF CHARLES DARWIN'S 200TH BIRTHDAY

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ABSTRACT. On February 12, 2009, the world celebrated the 200th birthday of Charles Darwin. His contributions to the study of evolution and human origins are well known, but his botanical research is underappreciated. Darwin published nine different books that focused on domesticated plants, insectivorous plants, climbing plants, and other botanical subjects, but his study on orchids is the most notable because it was the first book he published after the *Origin of Species* (1859). Darwin's book *On the Various Contrivances by which British and Foreign Orchids are Fertilised by Insects* (1862) was a systematic overview of both temperate and tropical orchid groups and their pollinators. The nine chapters treated members of Orchideae, Arethuseae, Neottieae, Vanilleae, Malaxideae, Epidendreae, Vandaeae, Cymbidieae (especially *Catasetum*), and Cyripedioideae. Orchid flowers were described and illustrated by Darwin in great detail, careful observations on pollinator behavior were recorded, and a healthy dose of speculation was presented. Our understanding of the phylogeny, pollination, physiology, and overall natural history of these orchid groups has advanced tremendously in the last 150 years. Few people realize that "the orchid book" is subtitled, ". . . and on the Good Effects of Intercrossing." That subject was of great concern to Darwin, and the orchids provided concrete examples necessary to substantiate his theory. Even today, orchids and Darwin's orchid book continue to inspire evolutionary biologists and exemplify the power of natural selection.

RESUMEN. En febrero 12 de 2009, el mundo celebró el 200avo cumpleaños de Charles Darwin. Sus contribuciones al estudio de la evolución y los orígenes del hombre son bien conocidos, pero su investigación botánica no ha sido apreciada en su justa medida. Darwin publicó nueve libros diferentes que se enfocaron sobre plantas domesticadas, plantas insectívoras plantas trepadoras, y otros temas botánicos, pero el más notable es su estudio sobre las orquídeas, ya que fue el primer libro publicado después del *Origen de las Especies* (1859). El libro de Darwin, *Sobre las estrategias por las cuales las orquídeas británicas y las introducidas son fertilizadas por insectos* (Darwin, 1862), fue una visión a la sistemática de las orquídeas de zonas templadas y tropicales y de sus polinizadores. Los nueve capítulos tratan especies de Orchideae, Arethuseae, Neottieae, Vanilleae, Malaxideae, Epidendreae, Vandaeae, Cymbidieae (especialmente *Catasetum*) y Cyripedioideae. Las flores de las orquídeas fueron descritas y estudiadas por Darwin con gran detalle, cuidadosos registros del comportamiento de los polinizadores fueron mantenidos, y una gran dosis de especulación fue agregada. Nuestra comprensión de la filogenia, polinización, fisiología, y toda la historia natural de estos grupos han avanzado tremendamente en los últimos 150 años. Pocas personas han notado que "el libro de las orquídeas", se subtitula... "y sobre el Buen Efecto del Entrecruzamiento". Fenómeno que fue de gran interés para Darwin y las orquídeas brindaron ejemplos concretos para substanciar su teoría. Aún en nuestros tiempos, el libro de "Orquídeas" de Darwin, continúa inspirando a los biólogos evolucionistas y ejemplifica el poder de la selección natural.

KEY WORDS: Charles Darwin, Orchidaceae, pollination

It was fitting that the Third International Conference on Andean Orchids was held in Ecuador in 2009. This country of only 256,371 square kilometers (98,985 square miles) boasts some of the most diverse

ecosystems on Earth – from the Amazon rain forest in the east across the Andes mountains and ultimately to the Galapagos Islands 972 km (604 miles) west of the Ecuadorian mainland. Indeed, because of their high

levels of biological diversity, the Andes have been called a cradle of evolution and the Galapagos the cradle of evolutionary theory. Why? Because from 1831-1836, the young English naturalist Charles Darwin sailed aboard the well-known expeditionary vessel, *HMS Beagle*, collecting flora and fauna along both the eastern and western coastlines of South America. It was during his time among the islands of the Galapagos, however, that Darwin made some of his most inspiring observations. These would ultimately serve to catalyze his theory of evolution by natural selection, which would eventually be detailed and published in 1859 — exactly 150 years ago. While the world celebrates the anniversary of *On the Origin of Species* (Darwin, 1859) this year, it also celebrates what would have been Darwin's 200th birthday (he was born February 12, 1809). For these reasons, Darwin's contributions to botany, and orchid biology in particular, are worthy of consideration this year, and it was all the more fitting to do so in Ecuador.

The young naturalist/botanist

Darwin's detailed descriptions of tortoises, finches, iguanas, and other vertebrate animals are well known, but the public is generally ignorant of Darwin's considerable contributions to plant biology. His interest in the "Vegetable Kingdom" must have developed at an early age and was only cultivated further by regular interactions with his teachers, friends, and colleagues who shared a similar passion for the natural sciences. Among the most influential scientists in Darwin's life were botanists, including John S. Henslow. Professor Henslow joined the faculty of Geology at Cambridge University in 1822 but five years later resigned from his position as Professor of Mineralogy after becoming Professor of Botany, a subject he held in higher regard and interest. Darwin owed much to Henslow for steering him into the study of natural history and also for introducing him to such well-respected botanists as Joseph Dalton Hooker, who would become his closest friend. Hooker married Professor Henslow's daughter and would eventually serve as Director of the Royal Botanic Gardens, Kew, for 20 years. At Kew, Charles Darwin eventually would be introduced by Hooker to the greatest American botanist of the 19th century,

Asa Gray, and the two men would develop a close professional relationship through regular written correspondence. There is no doubt that the shared passion for botany among all of these men cemented their friendships but would also certainly have had a direct influence on their independent intellectual pursuits.

Among the first pieces of evidence that we have to show how the study of plants may have influenced Darwin's construction of a case for evolution by natural selection is to be found in his *Journal and Remarks of the Voyage of the Beagle* (Darwin, 1839). Within the pages of this best-selling book it is well known that he documented the fact that different bird species inhabited different islands in the archipelago, each apparently adapted to its unique environment, but Darwin also documented the same phenomenon with some of the endemic plant species that he encountered:

If we now turn to the Flora, we shall find the aboriginal plants of the different islands wonderfully different... *Scalesia*, a remarkable arborescent genus of the Compositæ, is confined to the archipelago: it has six species; one from Chatham, one from Albemarle, one from Charles Island, two from James Island, and the sixth from one of the three latter islands: not one of these six species grows on any two islands. The species of the Compositæ are particularly local; in like manner we have seen that the different islands have their proper species of the mundane genus of tortoise, and of the widely distributed American genus of the mocking-thrush, as well as of two of the Galapageian sub-groups of finches.

It would not be for another 20 years that Darwin would make his greatest contribution to science by publishing *On The Origin of Species* (Darwin, 1859). Much has been written about this pivotal work, Darwin's most important, and the reader is encouraged to explore the book or at least to take a moment in order to reflect on its importance to human society. After being thrust into the spotlight of fame and controversy, however, Darwin did not end his basic research. In fact, he continued to publish until his death in 1882. It is generally underappreciated that during those intervening 23 years, most of Darwin's texts were focused on various aspects

of plant biology. They included books on insectivorous plants, climbing plants, plant movements, flowers, and orchids (see Table 1). In fact, the next book to be published immediately after the *Origin* was his 300-page treatise, *On the Various Contrivances by which*

British and Foreign Orchids are Fertilised by Insects (Darwin, 1862). The book contained 35 illustrations. While orchidologists today continue to find inspiration among the pages of this text, specialists in other disciplines such as the history of science have devoted considerable attention to the subtitle of the book, "... and on the Good Effects of Intercrossing".

TABLE 1. Charles Darwin's published books. Those with fairly strong or exclusively botanical themes are in boldface: 9 of 21=43%.

1839: <i>Journal and Remarks (The Voyage of the Beagle)</i>
1842: <i>The Structure and Distribution of Coral Reefs</i>
1844: <i>Geological Observations of Volcanic Islands</i>
1846: <i>Geological Observations on South America</i>
1849: <i>Geology from A Manual of Scientific Enquiry; Prepared for the Use of Her Majesty's Navy: and Adapted for Travellers in General.</i> , John F. W. Herschel, ed.
1851: <i>A Monograph of the Sub-class Cirripedia, with Figures of All the Species. The Lepadidae; or, Pedunculated Cirripedes.</i>
1851: <i>A Monograph on the Fossil Lepadidae; or, Pedunculated Cirripedes of Great Britain</i>
1854: <i>A Monograph of the Sub-class Cirripedia, with Figures of All the Species. The Balanidae (or Sessile Cirripedes); the Verrucidae, etc.</i>
1854: <i>A Monograph on the Fossil Balanidae and Verrucidae of Great Britain</i>
1858: <i>On the Tendency of Species to form Varieties; and on the Perpetuation of Varieties and Species by Natural Means of Selection</i> (Extract from an Unpublished Work on Species)
1859: <i>On the Origin of Species by Means of Natural Selection</i>
1862: <i>On the Various Contrivances by which British and Foreign Orchids are Fertilised by Insects</i>
1868: <i>Variation of Plants and Animals Under Domestication</i>
1871: <i>The Descent of Man, and Selection in Relation to Sex</i>
1872: <i>The Expression of Emotions in Man and Animals</i>
1875: <i>Movement and Habits of Climbing Plants</i>
1875: <i>Insectivorous Plants</i>
1876: <i>The Effects of Cross and Self Fertilisation in the Vegetable Kingdom</i>
1877: <i>The Different Forms of Flowers on Plants of the Same Species</i>
1880: <i>The Power of Movement in Plants</i>
1881: <i>The Formation of Vegetable Mould Through the Action of Worms</i>

Inbreeding and outcrossing

Darwin emphasized the roles of inbreeding and outcrossing in formulating his theory of evolution and would later write an entire book on the subject – *Effects of Cross and Self Fertilisation in the Vegetable Kingdom* (Darwin, 1876). After all, variation is the raw material of evolution by natural selection, and even though Darwin was not familiar with Mendelian genetics, he and others, especially livestock breeders, knew that sexual reproduction among unrelated individuals had the potential to produce a higher level of variation than did inbred lines. But Darwin's interest in the subject may have been more than academic. It has been argued (Moore, 2005) that he had a personal interest in the subject of inbreeding, for the Darwin and Wedgwood families had intermarried for several generations. Of the 62 aunts, uncles, and cousins born in the four generations founded by Charles Darwin's grandparents, Josiah and Sarah Wedgwood, 38 (61%) remained childless. Specifically, a total of 19 (73%) of the 26 children born from the first-cousin marriages in the "Darwood" family did not reproduce. Perhaps some of these couples chose to remain childless. Others may have lost children due to lack of modern medical care (although the families were both wealthy and would have had access to the best physicians of the day). It is likely, however, that this high level of infertility (i.e., reduced "fitness" in the sense of evolutionary theory) was due to genetic defects caused by inbreeding among the close relatives. Charles Darwin almost certainly recognized this fact. Even he married his first cousin, Emma Wedgwood, and several of their children died young (3 of 10) or suffered serious health problems. Only 3 of their 7 living children produced grandchildren for Charles and Emma. Could Darwin's personal family life have influenced the development of his theory of evolution with its emphasis on fitness defined by reproductive potential and descent from parent to offspring? Some believe that this was precisely the case (Moore, 2005).

Whatever the reason, Darwin went forth by publishing his orchid book to demonstrate that natural selection not only serves to explain animal evolution but plant evolution as well. One might assume that plants tend to self-pollinate (inbreed) more than do animals, because the former are stationary and their flowers generally hermaphroditic. If this were true, it would be a serious flaw in Darwin's theory. Thus, he argued that outcrossing among plants must be more common than perceived, and he used as examples the complex behavior of orchid pollinators coupled with detailed descriptions of orchid floral structure to demonstrate that these, and most likely other plants as well, are perfectly adapted to avoid selfing. In fact, when you think about it, what better group of organisms to document this fact than orchids, which have hermaphroditic flowers in which male and female organs are intimately united into one. This is certainly an extreme case that would appear at first glance to promote selfing if ever there was one! This fact was not lost on Darwin, who stated in the book that "unless we bear in mind the good effects which have been proved to follow in most cases from cross-fertilisation, it is an astonishing fact that the flowers of Orchids should not have been regularly self-fertilised. It apparently demonstrates that there must be something injurious in this latter process, of which I have elsewhere given direct proof." He concluded the orchid book stating that "it is hardly an exaggeration to say that Nature tells us, in the most emphatic manner, that she abhors perpetual self-fertilisation."

While the advantage of outcrossing is a theme that runs through the entire orchid book, the fact that Darwin published it immediately after the *Origin of Species* leads us to believe that he had other motives as well. In his rush to publish the *Origin* as quickly as possible (as what he called an unfinished "abstract"), Darwin was unable to provide all of the supporting material that he felt bolstered his argument. Critics were quick to attack, and the orchid book served as a partial response. Darwin wrote to his publisher, John Murray, on September 24, 1861, "I think this little volume will do good to the 'Origin', as it will show that I have worked hard at details." These detailed examples were provided not only for other naturalists but also for so-called natural theologians who were beginning to accept many of the facts presented by

geologists, paleontologists, and biologists in terms of the origins and antiquity of life but still saw God as the force guiding all laws of nature. Just as people today continue to debate the driving force of evolution either by means of natural selection or the hand of a creator, so they did as well in the mid-19th century as the *Origin* became a bestseller. Throughout the book Darwin dismisses what today might be called intelligent design in orchid flowers and instead provides example after example of what he regularly called "perfect adaptation." For example, Darwin felt no need to invoke the hand of God in referring to the flowers of *Spiranthes autumnalis* as a "perfect adaptation by which the pollen of a younger flower is carried to the stigma of an older flower." Asa Gray would later state that "if the Orchid-book (with a few trifling omissions) had appeared *before* the 'Origin' the author would have been canonised rather than anathematised by natural theologians."

Among the most celebrated of the detailed examples provided by Darwin in the orchid book is his hypothesis concerning the pollination of *Angraecum sesquipedale*. This orchid species endemic to Madagascar is often referred to as the "comet orchid" because of its unusually long nectar spur (the specific epithet of the species, *sesquipedale*, translates to 'a foot and a half'). Although the sphyngid moth pollinator of the comet orchid (*Xanthopan morgani praedicta*) would not be discovered until 1903, and in situ observations of visitation events would not be recorded on video until the early 21st century, Darwin's hypothesis for explaining the co-evolution between the orchid and insect would prove to be a classic example of the predictive power of evolutionary biology. In his own words, Darwin explained that "We can thus understand how the astonishing length of the nectary had been acquired by successive modifications . . . As certain moths of Madagascar became larger through natural selection in relation to their general conditions of life [or their proboscis became elongated] . . . those individual plants of the *Angraecum* which had the longest nectaries (and the nectary varies much in length in some Orchids) ... would be best fertilised. These plants yield the most seed, and the seedlings would generally inherit long nectaries; and so it would be in successive generations of the plant and the moth."

The orchid book, chapter by chapter

To this day, Darwin's orchid book remains a must-read for any botanist, and especially for those of us interested in orchidology. Yam *et al.* (2009) examined the influence of the book on aspects of orchid biology other than pollination ecology, such as physiology, structure, and taxonomy. They also provided a more detailed account of how the book came to be, and the reader who desires further information is encouraged to consult their paper. As a summary, however, I will present an overview of the book, chapter by chapter.

The first edition (Darwin, 1862) treated 63 genera of orchids, and included 34 illustrations dispersed among 365 pages of text. These were divided into seven chapters. By the time that edition sold out and a second edition was printed (Darwin, 1877; Fig. 1), Darwin had access to a great many more orchid taxa, especially from the Neotropics, Australia, and temperate North America. These included *Pterostylis*, *Caladenia*, *Thelymitra*, *Disa*, *Gongora*, *Sobralia*, *Pogonia*, *Platanthera*, and others. In total, the second edition was expanded to treat 85 different genera of orchids (a 35% increase), four additional illustrations were provided, and the chapters were reorganized into nine. For the most part, these were arranged according to the accepted classification system of the day as proposed by John Lindley (1827). Subfamilies were not considered, but Orchidaceae (excluding apostasioid orchids) were divided into tribes.

Chapters 1 and 2 treat Ophreae, in particular the European terrestrial orchids such as *Orchis* and *Ophrys*, the bee orchids. A total of 73 pages were devoted to detailed observations and experimentation with the orchids with which Darwin would have been most familiar because they grew near his home in the English countryside. Among the genera considered are *Orchis*, *Ophrys*, *Herminium*, *Peristylus*, *Gymnadenia*, *Platanthera*, *Habenaria*, *Disa*, and *Bonatea*.

Chapter 3 and 4 are short and consider tribes Arethuseae and Neottieae, respectively. Although *Vanilla* is recognized today as only distantly related to these lower epidendroid groups, it was treated among the 12 pages of Chapter 3. Among the other genera discussed here are *Cephalanthera*, *Sobralia*, *Pterostylis*, *Caladenia*, and *Pogonia*. More recent systems of classification, especially those based on DNA evidence

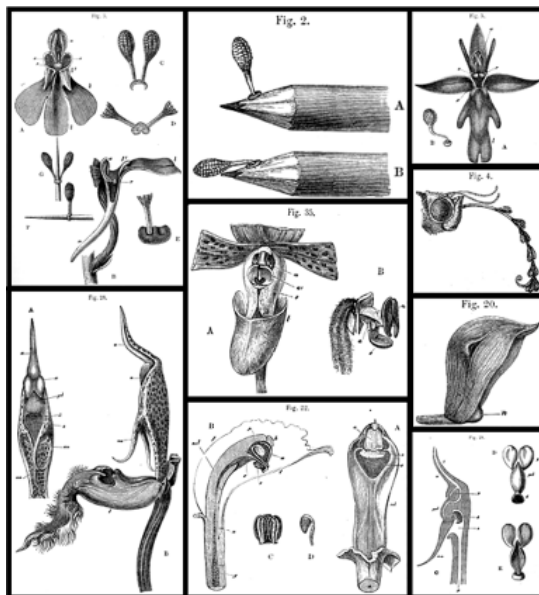


FIGURE 1. A selection of nine original figures from Charles Darwin's (1877) *The Various Contrivances by which Orchids are Fertilised by Insects*. 2nd edition. John Murray, London.

(e.g., Chase *et al.*, 2003) have demonstrated that these orchids, which share plesiomorphic aspects of their floral morphology, actually belong to three different subfamilies. Likewise, many of the genera considered together in Lindley's concept of tribe Neottieae and discussed by Darwin in Chapter 4 (*Epipactis*, *Listera*, *Neottia*, *Epipogium*, *Goodyera*, *Spiranthes*, *Thelymitra*) are now classified among different tribes of subfamilies Orchidoideae and Epidendroideae. Darwin devoted 34 pages to them.

The subsequent chapters of the orchid book treated those orchids classified within Epidendroideae, the largest subfamily of Orchidaceae. Today we count nearly 20,000 different species in that group. However, in Darwin's day less than 2000 orchid species were recognized in total. It is almost humorous to note that Lindley estimated that there might be as many of 6000 orchid species to be discovered eventually. It might not be surprising, therefore, to realize that Darwin only devoted 20 pages of text in Chapter 5 to tribes Malaxaeae and Epidendreae. These are primarily tropical orchid tribes, and many of the most species-rich genera were still poorly collected, especially those from higher elevations of the Andes, New Guinea, and southeast

Asia. Among the genera that were known to Darwin – primarily through relationships with horticulturists at Kew and from other glasshouse collections – were *Malaxis*, *Bulbophyllum*, *Dendrobium*, *Cattleya*, *Laelia*, *Epidendrum*, *Pleurothallis*, and *Stelis*. Darwin was intrigued by the odd flowers of *Zootrophion atropurpureum*, which he knew and illustrated in Figure 20 as *Masdevallia fenestrata*. The taxonomy of the orchids treated by Darwin has changed considerably over the past century and is presented below as an Appendix. The names as they appeared in the second edition of the orchid book as well as their currently accepted names are provided for comparison.

Following in sequence we reach Chapters 6 and 7, which are 74 pages in length and devoted to the plants Darwin said were “the most remarkable of all orchids.” These are members of tribe Vandeeae (as then understood), and particular emphasis was given to *Catasetum* and its fast-action mechanism of pollinarium ejection. This is not surprising because Darwin was obviously fascinated by plant movement. In 1875, he published *Movement and Habits of Climbing Plants*. That same year, his *Insectivorous Plants* considered the movement of sundews (*Drosera* spp.) and Venus’ flytrap (*Dionaea muscipula* Ellis). In 1880, he published *The Power of Movement in Plants*. Other orchids discussed in chapters 6 and 7 of the orchid book are *Calanthe*, *Miltonia*, *Sarcanthus*, *Maxillaria*, *Angraecum*, *Coryanthes*, *Mormodes*, and *Cycnoches*.

Chapter 8 is devoted to the lady’s-slipper orchids, with one genus (*Cypripedium*) at that time classified into tribe Cyripedieae. Among the 20 pages is also found a discussion concerning the homologies of orchid floral organs. This is a wonderful chapter in which Darwin demonstrated his skill as a keen observer and experimental biologist. For example, he described several attempts to introduce various insects to the flowers of *Cypripedium calceolus* in order to record their behavior, only to find that some “were either too large or too stupid, and did not crawl out properly.” Originally he felt sure that lady’s slipper orchids were pollinated when “insects alighted on the labellum and inserted their proboscides through either of the orifices close to the anthers,” but later realized that this made little sense given that “if an insect were to insert its proboscis, as I had supposed, from the outside through

one of the orifices, . . . the stigma would be liable to be fertilized by the plant’s own pollen.” This would be contrary to the argument made throughout the book in favor of mechanisms to ensure cross-fertilization, and so he was delighted to document that his experiments proved just the opposite — *Cypripedium* also manages to avoid self-pollination.

An example of a hypothesis presented in the book that would eventually be proven incorrect, however, can be found in this chapter. Figure 36 in the book shows an artificial section through a monandrous orchid flower. At the time it was assumed that three anthers of an ancestral inner androecial whorl were modified to form the orchid clinandrium and apex of the column. One of the anthers from the outer whorl remained fertile, leaving two others “of the same whorl combined with the lower petal, forming the labellum.” Today the structure and homologies of the orchid labellum are not viewed in this way. There is no evidence that sterile stamens or anthers are involved in its construction.

Finally, in Chapter 9, titled ‘Gradation of Organs, etc. & Concluding Remarks’, Darwin provides a summary of the various examples he has presented throughout the book, and makes his final arguments. He states, “it may naturally be inquired, Why do the Orchideae exhibit so many perfect contrivances for their fertilization? From the observations of various botanists and my own, I am sure that many other plants offer analogous adaptation of high perfection; but it seems that they are really more numerous and perfect with the Orchideae than with most other plants.” From that statement alone it is clear that Charles Darwin had a passion for orchids. One might even say that he contracted what today some would call orchid fever! During in his life he would write to colleagues such statements as “you cannot conceive how the orchids have delighted me” (Darwin, 1861a), “I am sillily and very idly interested in them” (1860), “the orchids are more play than real work”, and “the orchids have been a splendid sport” (Darwin, 1862b). This highly respected and influential scientist, a man who was passionate about animal and plant biology and who would do so much to advance evolutionary theory, would state that “orchids have interested me more than almost anything in my life” (Darwin, 1861b). Many of us today feel the same and should feel pride, but also humility, in following his footsteps.

APPENDIX

Genera and species of Orchidaceae referenced in Darwin (1877). Page numbers are provided based on this edition, and spellings are reproduced exactly as they appear within the index of the book. Names within brackets are those accepted currently by the World Checklist of Monocotyledons. (2009). The Board of Trustees of the Royal Botanic Gardens, Kew. Published on the Internet; <http://www.kew.org/wcsp/monocots/> accessed 2 August 2009.

- Aceras anthropophora*, 26, 258 [*Orchis anthropophora*] — *tridentatum*, 191, 196, 197, 239, 256, 269 [*Catasetum macrocarpum*]
 — *longibracteata*, 26 [*Barlia robertiana*]
Acianthus exsertus, 90
 — *fornicatus*, 90, 280
 — *sinclairii*, 90, 280
Acontia luctuosa, 31 [*Acronia luctuosa* = *Pleurothallis luctuosa*]
Acropera, 154, 156, 276 [*Gongora*]
 — *loddigesii*, 166 [*Gongora galeata*]
 — *luteola*, 166, 239 [*Gongora galeata*]
Aerides, 156, 265
 — *cornutum*, 265 [*Aerides odorata*]
 — *odorata*, 158
 — *virens*, 156 [*Aerides odorata*]
Angræcum, 251
 — *distichum*, 154
 — *eburneum*, 155
 — *sesquipedale*, 154, 162, 282, 265
Apostasia, 248
Barkeria, 146
Bulbophyllum, 274, 276 [*Bulbophyllum*]
 — *barbigerum*, 138
 — *cocoinum*, 137
 — *cupreum*, 137, 265
 — *rhizophoræ*, 137 [*Bulbophyllum falcatum* var. *velutinum*]
Bonatea speciosa, 71, 76, 244, 264, 361
Brassia, 156
Caladenia dimorpha, 89
Calæna, 89 [*Caleana*]
Calanthe dominii, 161 [*Calanthe x dominii*]
 — *masuca*, 161, 267, 269 [*Calanthe sylvatica*]
 — *veratrifolia*, 280 [*Calanthe triplicata*]
 — *vestita*, 162
Catasetum, 256, 270
 — *callosum*, 192, 195
 — *luridum*, 191
 — *mentosum*, 206
 — *planiceps*, 193
 — *saccatum*, 180–185, 239
 — *tabulare*, 192
 — *tridentatum*, 191, 196, 197, 239, 256, 269 [*Catasetum macrocarpum*]
Cattleya, 143–148, 239, 265
 — *crispa*, 147 [*Sophronitis crispa*]
Cephalanthera, 277
 — *ensifolia*, 86 [*Cephalanthera longifolia*]
 — *grandiflora*, 80–86, 239, 242, 249, 259, 269, 277, 287, 290 [*Cephalanthera longifolia*]
Chysis, 146
Cirrhaea, 171
Coelogyne cristata, 146
Coryanthes, 90, 173, 232, 265
 — *fieldingii*, 175
 — *macrantha*, 175
 — *speciosa*, 174
 — *triloba*, 281 [?]
Cycnoches egertonianum, 224
 — *ventricosum*, 220–224
Cymbidium giganteum, 155, 252, 260, 263 [*Cymbidium iridiodes*]
Cypripedium, 226, 229, 262, 275
 — *acaule*, 229
 — *barbatum*, 239 [*Paphiopedilum barbatum*]
 — *calceolus*, 229–231, 282
 — *candidum*, 235
 — *pubescens*, 229, 230 [*Cypripedium parviflorum* var. *pubescens*]
 — *purpuratum*, 239 [*Paphiopedilum purpuratum*]
Cyrtostylis, 90
Dendrobium, 287
 — *bigibbum*, 142
 — *chrysanthum*, 138–142, 265
 — *cretaceum*, 142, 291 [*Dendrobium polyanthum*]
 — *formosum*, 142
 — *speciosum*, 281
 — *tortile*, 142
Disa, 265
 — *cornuta*, 78
 — *grandiflora*, 77, 281 [*Disa uniflora*]
 — *macrantha*, 78, 290 [*Disa cornuta*]
Disperis, 265

- Epidendrum cochleatum*, 249 [*Prosthechea cochleata*]
 — *floribundum*, 146, 249 [*Epidendrum paniculatum*]
 — *glaucum*, 146 [*Dichaea glauca*]
Epipactis, 239, 251
 — *latifolia*, 100, 101, 259, 282, 287 [*Epipactis helleborine*]
 — *microphylla*, 102
 — *palustris*, 93–100, 275
 — *purpurata*, 102
 — *rubiginosa*, 102 [*Epipactis atrorubens*]
 — *viridiflora*, 102, 291 [*Epipactis purpurata*]
Epipogium gmelini, 103 [*Epipogium aphyllum*]
Eulophia viridis, 156, 269 [? *Eulophia viridiflora* = *Eulophia epidendreae*]
Evelyna, 265 [*Elleanthus*]
 — *carinata*, 146, 239, 241 [*Elleanthus carinata*]
Galeandra funkii, 155 [*Galeandra baueri*]
Glossodia, 237
Gongora, 276
 — *atropurpurea*, 169
 — *maculata*, 168
 — *truncata*, 169
Goodyera, 239, 260
 — *discolor*, 105 [*Ludisia discolor*]
Goodyera pubescens, 105
 — *repens*, 103, 105
Gymnadenia, 251
 — *albida*, 43, 68 [*Pseudorchis albida*]
 — *conopsea*, 32, 40, 43, 65, 238, 239, 255, 271, 272
 — *odoratissima*, 68
 — *tridentata*, 68, 291 [*Platanthera clavellata*]
Habenaria bifolia, 78, 40, 43, 251 [*Platanthera bifolia*]
Habenaria chlorantha, 43, 69, 239, 244, 251 [*Habenaria viridiflora*]
Herminium monorchis, 59, 61, 255
Laelia, 146
 — *cinnabarina*, 148 [*Sophranitis cinnabarina*]
Leptotes, 146
Liparis pendula, 239, 241 [*Stichorkis viridiflora*]
Listera, 251, 287 [*Neottia*]
 — *cordata*, 124 [*Neottia cordata*]
 — *ovata*, 115–124, 276 [*Neottia ovata*]
Lycaste skinneri, 155, 260
Malaxis, 251, 276
 — *paludosa*, 32, 129–135, 239, 241, 241, 258, 284 [*Hammarbya paludosa*]
Masdevallia, 241, 274, 276
 — *fenestrata*, 135, 136, 142 [*Zootrophion fenestratus*]
Maxillaria, 156, 278
 — *ornithorhyncha*, 157, 159 [?]
Megaclinium falcatum, 138 [*Bulbophyllum falcatum*]
Microstylus rhedii, 132, 135 [*Malaxis resupinata*]
Miltonia clowesii, 154, 155
Monachanthus viridis, 196, 197, 198, 201 [*Catasetum cernuum*]
Mormodes ignea, 208–219, 249, 276, 283
 — *luxata*, 219
Myanthes barbatus, 192, 199, 203, 205 [*Catasetum barbatum*]
Neotinia intacta, 27, 291 [*Neotinea maculata*]
Neottia nidus-avis, 125, 258, 290
Nigritella angustifolia, 27 [*Gymnadenia nigra*]
Notylia, 171
Odontoglossum, 156
Oncidium, 153, 156, 158, 239, 251, 266
 — *unguiculatum*, 252
Ophrys apifera, 52, 54–58, 259, 279, 291
 — *arahnites*, 51 [*Ophrys apifera*]
 — *aranifera*, 50, 280 [*Ophrys sphegodes*]
 — *muscifera*, 32, 45, 49, 280 [*Ophrys insectifera*]
 — *scolopax*, 52, 292
Orchis fusca, 15, 35, 37 [*Orchis purpurea*]
 — *hircina*, 25, 39, 273 [*Himantoglossum hircinum*]
 — *latifolia*, 15, 35, 37, 255 [*Dactylorhiza incarnata*]
 — *maculata*, 15, 34, 32, 35, 37, 39, 255, 255, 277, 278 [*Dactylorhiza maculata*]
Orchis mascula, 6, 273, 278
 — *militaris*, 36, 37
 — *morio*, 15, 128, 33, 37, 39, 278 [*Anacamptis morio*]
 — *pyramidalis*, 16, 21, 34, 37, 39, 38, 254, 256, 260, 261, 264, 272, 273 [*Anacamptis pyramidalis*]
 — *ustulata*, 25 [*Neotinea ustulata*]
Ornithocephalus, 160
Peristylus viridis, 43, 63, 255 [*Coeloglossum viride*]
Phaius, 146
 — *grandifolius*, 280 [*Phaius tankervilleae*]
Phalaenopsis, 153, 159, 276
 — *amabilis*, 159
 — *grandiflora*, 159, 269 [*Phalaenopsis amabilis*]
Platanthera, 75
 — *chlorantha*, 69
 — *dilatata*, 77 [*Piperia dilatata*]
 — *flava*, 76, 77
 — *hookeri*, 75

- *hyperborea*, 76, 291
Pleurothallis ligulata, 135 [*Stelis ligulata*]
— *prolifera*, 135 [*Acianthera prolifera*]
Pogonia ophioglossoides, 86
Pterostylis, 232
— *longiflora*, 87, 89 [? *Pterostylis longifolia*]
— *trullifolia*, 86, 88, 280
Rodriguezia secunda, 159 [*Rodriguezia lanceolata*]
— *suaveolens*, 156, 159 [*Gomesa foliosa*]
Saccolabium, 153, 156
Sarcanthus, 276 [*Cleisostoma*]
— *parishii*, 142 [*Cleisostoma parishii*]
— *teretifolius*, 154, 156, 268 [*Cleisostoma simondsii*]
Selenipedium palmifolium, 232
Serapias cordigera, 27
Sobralia macrantha, 91
Sophronitis, 146
Spiranthes australis, 114, 275, 291 [*Spiranthes sinensis*]
— *autumnalis*, 106-114, 239
— *cernua*, 111
— *gracilis*, 111 [*Chlorosa gracilis*]
Stanhopea, 155, 276
— *devoniensis*, 171 [*Stanhopea hernandezii*]
— *oculata*, 171
Stelis, 274
— *racemiflora*, 135 [*Stelis quadrifida*]
Thelymitra, 291
— *carnea*, 127, 280
— *longiflora*, 127 [? *Thelymitra longifolia*]
Uropedium, 240 [*Phragmipedium*]
Vanilla aromatica, 90 [*Vanilla planifolia*]
Warrea, 155, 270
Zygopetalum mackai, 155 [*Zygopetalum maculatum*]

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ORCHIDS IN A CHANGING CLIMATE

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ABSTRACT. Orchids have a long and distinguished recorded history, traceable back to the ancient Greeks. For two millennia or more, our knowledge of orchids remained sketchy, mainly because the main centers of learning were in temperate regions with poor orchid floras. Beginning with the Renaissance, knowledge increased more rapidly. Almost half a century ago, Professor William Stearn outlined this progress in his landmark lecture at the Third World Orchid Conference in London. However, knowledge has moved rapidly since then. In this lecture, I would like to update Stearn's story. The availability of new technologies has produced unprecedented advances in many aspects of orchids from our understanding of the origins of orchids to dealing with threats to their future survival. The world has become increasingly aware of issues such as climate change that are likely to have a dramatic effect on the world's orchids. I have identified five developments that have underpinned these new insights since Stearn's lecture was delivered: • Cloning orchids; • Computing power; • DNA analysis; • The fossil orchid; • Conservation techniques.

RESUMEN. Las orquídeas gozan de una historia registrada larga y distinguida, que puede ser rastreada a los antiguos griegos. Durante dos milenios o más, nuestro conocimiento relativo a las orquídeas se ha mantenido superficial, básicamente debido a que los centros de conocimientos se encontraban en regiones templadas con una pobre flora de orquídeas. Al inicio del Renacimiento, el conocimiento aumentó en forma más rápida. Hace casi medio siglo, el profesor William Stearn delineó este progreso en su conferencia que constituye un hito, presentada en la Tercera Conferencia Mundial de Orquídeas en Londres. Sin embargo, el conocimiento se ha desplazado en forma vertiginosa desde ese momento, y ha identificado cinco desarrollos principales que han dado sustento a estos nuevos discernimientos – clonación de orquídeas, potencia de computación, análisis de ADN, la orquídea fósil y técnicas de conservación – que ha generado impactos principales en la ciencia de las orquídeas, horticultura, y conservación. La disponibilidad de nuevas tecnologías y descubrimientos han generado avances sin precedentes en muchos aspectos relativos a las orquídeas, desde nuestra comprensión relativa a los orígenes de orquídeas al manejo de las amenazas para su supervivencia futura. El mundo se ha vuelto crecientemente consciente de temas como el cambio climático que con gran probabilidad van a tener un efecto dramático sobre las orquídeas del mundo.

KEY WORDS: Orchids, cloning, computing, DNA analysis, fossil orchid, conservation techniques

Almost half a century ago, the late William Stearn (1960), addressing the 3rd World Orchid Conference in London, presented what he considered to be 10 landmarks in the knowledge of orchids, a family which he labeled as the most promiscuous of all plants based on the ability of often unrelated species to produce successful hybrids. His landmarks were as follows:

1. The first naming of orchids by ancient Greeks and Romans dating from before the time of Christ.
2. The first recognition of orchids as a special group in late 17th century.
3. The introduction and first flowering tropical

orchids to Europe in the late 17th and early 18th centuries.

4. The application of binomial nomenclature to orchids by Linnaeus in 1753.
5. The change in method for heating greenhouses from dry to wet heat, stimulating their introduction and the publication of finely illustrated books about them in the 19th century.
6. The elucidation of the pollination mechanisms of orchids by Darwin.
7. The raising and flowering of the first artificial hybrid in 1861.

8. The raising and flowering of the first artificial intergeneric hybrid in 1868.
9. The discovery of the orchid mycorrhizal association.
10. The raising of the first orchid seedlings aymbiotically in the early 20th century.

In this lecture I would like to bring Stearn's landmarks up to date by suggesting five new landmarks since his comprehensive historical survey of the orchids. It is perhaps symptomatic of the speed of change over the past few years that I could easily list many more. The new major landmarks I would submit are as follows:

- Micropropagation: The cloning of orchids, which has made them available to all, leading to a truly worldwide trade in orchid hybrids.
- Computing: The application of computing power for orchids and the publication of the first world checklist of orchids on the internet.
- DNA analysis: The application of DNA analysis to orchid systematic problems, including estimating the age of the family. Publication of *Genera Orchidacearum*, introducing a new system of classification for the orchids, incorporating molecular and morphological data.
- The fossil record: The first irrefutable orchid fossil, allowing dating of the origins of the family.
- Conservation: A new awareness of the fragility of orchids and their habitats and the need for their conservation both *in situ* and *ex situ*, a situation made more urgent by the threat of climate change.

I will deal with each in turn, some in more detail than others. Some of the landmarks have produced wholly positive outcomes, but others have been met with controversy.

Micropropagation

Cloning orchids by meristem culture is so commonplace nowadays that we tend to forget what a revolution it has nurtured. Orchids have been transformed from the playthings of the rich to an everyday commodity, admittedly a fashionable one. Every department store and greengrocer now sells cut-flower and pot-plant orchids, and prices have tumbled. Consequently, the public's appreciation of orchids has improved, and the status of the orchid as the most

charismatic of plants has been bolstered.

Not all the consequences have been beneficial to the core orchid community. Orchids are now commonly sold without their correct name. Plants are marketed as 'moth orchid', 'windowsill orchid' or other trivial names. When a generic name is given, it is often without a grex or clonal name. The introduction of plant patents some years ago has further confused buyers but benefited breeders. Mutations can also arise in culture, requiring new cultivar names to be applied to those clones that differ from the parental plant. The problem does not, of course, affect the buyer whose interest in the plant is purely decorative, but good clones have been multiplied and the offspring used to produce new hybrids. If the parental names are absent or incorrect, the offspring cannot be named according to longstanding rules for naming of cultivated plants (Brickell, 2004). It could be argued that names are unimportant, but, as I will elaborate upon later, they form the backbone of access to knowledge about orchids, indeed about all organisms.

Computing

It is perhaps self-evident that computers have changed the world. I would like to consider how they have changed orchid science and culture through a few examples. The number of websites now devoted to orchids is immense. You can buy orchids over the internet, find out how to identify them, name them, grow them, propagate them, and conserve them all at the click of a button. Input the word 'orchid' into a web-search provider, and pages of addresses are revealed. Some sites, such as Wikipedia, have developed a holistic approach, but the quality of information on orchids in them is variable. Websites run by the American Orchid Society (www.aos.org) and Royal Horticultural Society (http://www.rhs.org.uk/plants/plant_groups/orchids.asp) are authoritative and contain high quality information and images on many aspects of orchids. A number of societies and orchid groups also publish their journals and newsletters on the web.

Original high-quality information on orchids can also be sought on a number of other websites. I would like to feature two here, the first of which is the World Monocot Checklist (<http://www.kew.org/wcsp/monocots>) based at the Royal Botanic Gardens, Kew. It is regularly updated through an international network

of orchid specialists who provide comments and queries on the taxonomy used by its compiler, Rafael Govaerts. Why is a checklist of orchids important? We cannot communicate satisfactorily about anything unless we can give it a ‘handle’. For living organisms, their scientific names provide the handles for knowledge to be shared. Orchid names and their synonyms provide the only reliable spine to access information on orchids.

The second website I would recommend and use regularly is that being developed by the Jany Renz Foundation at the University of Basel (<http://orchid.unibas.ch/site/herbarium.php>). Features of this website include thousands of images of orchids (photographs, illustrations from all of the historically important orchid books, and herbarium specimens) and access to the most complete bibliography of the orchids (BibliOrchidea). A searchable biographic database of all of the most significant orchid personalities is another useful feature.

DNA analysis

For the scientist, one of the most significant features of the computer is its ability to analyze large data sets, and this brings us to the next landmark event, the analysis of orchid DNA. DNA data sets can be large, especially when several genetic sequences are analyzed at once. It would not be an exaggeration to state that the ability to sequence the DNA of orchids has revolutionized our understanding of the family. The work of Mark Chase and his many collaborators has resulted in the evolutionary history of orchids becoming the best understood of any family of flowering plants. This is in marked contrast to the position just 25 years ago when few scientists were attracted to the family, which was considered to be too large and too horticultural to be of interest. In short, most scientists preferred to work on smaller families where their results were less likely to be the subject of horticultural dispute and infighting. This situation has fortunately changed dramatically. Chase’s work has attracted the attention, interest, and collaboration of some of the brightest young scientists. Their work has led to a better understanding of orchids as a family, the relations of its constituent parts, and the classification of orchids.

We now know that:

1) The closest relatives of orchids are a small number of mainly Southern Hemisphere families of

asparagoid monocots (Table 1), of which the best known is *Hypoxis*, a terrestrial genus with plicate leaves and yellow stellate flowers.

- 2) Orchids are a monophyletic family that includes apostasioids, cyripedioids, and the rest of the orchids as defined by Willis (1973). Both apostasioids and cyripedioids have been recently considered discrete families by some authors (e.g. Rasmussen, 1985).
- 3) *Vanilla* and its relatives (16 genera in all) are an ancient lineage worthy of subfamilial status.
- 4) Spiranthoid orchids are not worthy of subfamilial status and comprise a group within the orchidoids.
- 5) The circumscriptions of many long-accepted genera, e.g. *Cattleya*, *Laelia*, *Masdevallia*, and *Oncidium* have been greatly amended. Others, notably *Odontoglossum*, do not warrant recognition at all.
- 6) Floral features have often misled taxonomists, whereas vegetative characters can be more conservative and better reflect relationships, an idea first proposed by Pfitzer over a century ago.

These ideas are currently being assembled in the monumental *Genera Orchidacearum* (Pridgeon *et al.*, 1999, 2001, 2003, 2005), one volume of which still remains. This work has involved close to 100 collaborators worldwide. It is not, and was never meant to be, the final word on orchids and their classification. However, it does summarize our current knowledge of the phylogeny (evolutionary relationships), classification, and many other aspects of the family.

DNA has also been used at the species and infraspecific levels. One interesting project with wider

TABLE 1. Families of Asparagales allied to Orchidaceae (Chase, 2001).

Family	Distribution
Asteliaceae	Australia
Boryaceae	Australia
Doryanthaceae	Australia
Blandfordiaceae	Australia
Lanaceae	South Africa, South America
Hypoxidaceae	Africa, South America
Tecophilaceae	South Africa, South America
Ixioliriaceae	Asia

implications is the Darwin Initiative-sponsored project in Costa Rica, run by Vincent Savolainen and Jorge Warner, which is seeking to barcode the 1300 or so orchid species found in the country. The consequences of this for an understanding of species delimitation, rapid identification, and conservation have only begun to be considered.

In Australia the use of DNA sequencing for orchid conservation is being rapidly developed by Kingsley Dixon and his team at King's Park, Perth, Australia. Recent work has indicated that the two surviving populations of the Western Australian underground orchid (*Rhizanthella gardneri*) might represent two closely allied but distinct species (Kingsley Dixon, personal communication). Cryptic species have also been identified using DNA sequencing in other Australian orchid genera, notably *Drakaea* (Hopper and Brown, 2007) and *Chiloglottis* (Florian Schiestl, personal communication).

Conversely, DNA analysis of the 259 currently accepted European *Ophrys* species has revealed only 10 distinct groups separable by their DNA. It seems probable that many new species are described where there are many botanists rather than where biodiversity is greatest (Dion Devey, personal communication).

The other major issue with the new classification is the changing of generic concepts from long-accepted ones. This affects a number of the most important genera in horticulture, including *Cattleya*, *Laelia*, *Masdevallia*, *Odontoglossum*, and *Oncidium*. In the era before DNA analysis, the orchid registrar used a system of horticultural equivalents to conserve well-established generic and specific names for the orchid hybrid register. These names survived for decades in horticultural use when the botanists had long since consigned them to synonymy. For the past few years or so, the system has been abandoned, and the currently accepted scientific names have been used by the registrar, leading to many changes not only in specific names but also in hybrid generic (nothogeneric) names. The rationale for this is that the new DNA-based classification better reflects true affinities and breeding behavior and that the use of computers allows the ready retrieval of both the old and new names, obviating the need for horticultural equivalents. For the most part, the Registrar has made changes only where the scientific evidence is sound

and a degree of consensus among his advisors has been achieved. However, phylogenies are subject to different interpretation, so there is plenty of room for disagreement. My own opinion is that the present system of nothogeneric recognition is no longer useful in orchid hybridizing and is often confusing. Most nothogeneric names have little information content, particularly those for trigeneric hybrids and above which use a personal surname followed by -ara. I believe that a new system that recognizes the breeding groups is necessary to prevent confusion. Such a system will simplify registration and label writing as long as grex names are not repeated within a breeding group (which they mostly are not!). Mark Chase, Sarah Thomas, and I spelled out the need for a new system some years ago (Cribb *et al.*, 1999).

The orchid fossil record

The discovery of the first irrefutable orchid fossil, *Meliorchis caribea*, was announced by Ramirez *et al.* (2007) in the journal *Nature*. It comprised an orchid pollinarium on the back of a bee, the extinct *Proplebeia dominicana*, set in 15-20 million-year-old Miocene amber from the Dominican Republic. The discovery of the fossil represents a significant step forward in our knowledge of the antiquity of the orchids. The pollinarium can be safely assigned to a species of the terrestrial Goodyerinae, possibly *Kreodanthus* or *Microchilus*, probably no longer extant but having living relatives. The significance of this discovery confirms that the orchids are an ancient group, a view developed from the DNA work where the divergence of particular orchid taxa can be estimated from the rate at which nucleotide changes accumulate in DNA sequences. Chase (2001) suggested that the orchid lineage might be up to 90 million years old, in contrast to earlier opinions that the family evolved recently (Schmid & Schmid, 1977; Labandeira, 1998). Ramirez *et al.* (2007) suggested a date of about 76-84 million years ago in the late Cretaceous for the emergence of the family. Both support a pattern of an ancient family that contains five surviving lineages of which three — the apostasioids, cypridoids, and vanilloids — are now represented by relatively few surviving species. In contrast, the predominantly terrestrial orchidoids and the mainly epiphytic epidendroids have been extremely successful with a rapid adaptive radiation in relatively

recent times, particularly since the end of the last glaciation in the tropical mountain chains, such as the Andes, Central American highlands, the Himalayas, and the mountains of the Malay Archipelago.

Conservation

The orchid world can be proud of its considerable effort towards orchid conservation, particularly in the last 20 years. It has been aware of the rarity of many species, especially some of the showiest orchids, for over a century. In the 1880s, H. G. Reichenbach expressed concern at the scale of orchid collection for the nursery trade. Over the past 25 years, the rapidly increasing rate at which orchids and their habitats have disappeared has added impetus to a number of initiatives by the orchid community that have begun to address these serious issues. The causes are well documented, but the initiatives are perhaps less well appreciated by the public at large.

The Orchid Specialist Group, an arm of IUCN (The World Conservation Union) Species Survival Commission, has been particularly active and successful in stimulating research and projects on endangered orchids. The OSG comprises some 200 orchid scientists and horticulturists worldwide. Under the chairmanship of Michael Fay of the Royal Botanic Gardens, Kew, it has sponsored three successful International Orchid Conservation Conferences, in Australia, USA, and Costa Rica. The fourth is due in 2011 and will be held in the Czech Republic. Further information can be gained from the OSG website (<http://www.orchidconservation.org/osg>). The OSG also produces an electronic newsletter.

Perhaps the main result of the work of the OSG has been to bring together current ideas and methodologies for orchid conservation, both in situ and ex situ, and emphasize the need for the integration of both approaches to attempts to conserve orchids. One of the main products of the First Conference in Perth, Australia, was a techniques manual entitled *Orchid Conservation* (Dixon *et al.*, 2003).

In 2003, the OSG established a charitable foundation named Orchid Conservation International (<http://www.orchidconservation.org>) to support its work and that of orchid conservation projects worldwide. This is one of several successful grant-giving bodies, ranging from the American Orchid Society and Australian Orchid

Foundation to the San Diego Orchid Society and 1% for Orchid Conservation.

On a broader scale, I would like to mention the recent Darwin Initiative project to set up a world network of orchid species seed-banks. The first two workshops in Chengdu, China, and Quito, Ecuador, attracted a broad-based response. The project, Orchid Seed Stores for Sustainable Use, aims to establish protocols for orchid seed collection and storage based upon sound scientific evidence and set up a network of active orchid seed-banks in orchid-rich countries. [See paper by Seaton and Pritchard in this volume. – ed.]

Orchids face increasing threats to their existence, not only the obvious ones posed by increasing human population, logging, mining, and exploitation. Climate change will undoubtedly affect orchids. David Roberts (personal communication) has shown that orchid flowering times in the UK are increasingly out of synchronization with the emergence of their pollinating insects. Many naturalists have noted the decrease and disappearance of orchids from local habitats that appear to be still suitable. Was this triggered by climate change? It may well have been, because orchids have complex interactions with their environment that can easily be upset, from the fungi and bacteria that control germination and early growth to the pollinators that are necessary to produce viable seed. Change in one factor can wreck such sensitive interactions, and all the evidence indicates that climate change will be a powerful driver of irreversible change (Intergovernmental Panel on Climate Change Report – IPCC, 2007).

Conclusions

Rapid development of techniques over the past 50 years has meant that our knowledge of orchids has increased at a rate far greater than at any time in the past. New techniques have brought new and exciting talent into orchids in the fields of science, horticulture, and conservation. Not all of the developments have been welcome in the orchid community, particularly those that require the relearning of plant relationships, classification, and names. Old ideas and concepts are being ditched and new ones proposed with frightening speed. With sound scientific information and analysis, we can take some new concepts happily on board, whereas for others the evidence remains shaky. The

science of orchid conservation can also provide a more secure future for orchids if it is applied more widely. We have the knowledge and technology to conserve orchids, but often the limiting factor is funding. The new Darwin Initiative on global seed-banking of orchids is one that is good news for orchids and orchid growers, providing access to plants that can no longer be found in the wild or are protected and cannot be taken from it. Overshadowing all this, however, is the unknown effect of global climate change predicted by the recent report from a UN panel of experts. Will orchid habitats survive the upheavals that have been predicted? I do not know, but I would love to be around to hear the update on orchid landmarks in 50 years.

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¿LAS SOBRALIAS SE PUEDEN CLASIFICAR? — EL COMPLEJO DE *SOBRALIA WARSZEWICZII*

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RESUMEN. *Sobralia warszewiczii* Rchb.f. fue descrita de Panamá occidental en 1852. El nombre se ha usado hasta el sur de México, aunque ninguna de las especies con flores lilas o moradas conocidas de Costa Rica concuerda del todo con *S. warszewiczii*. Una especie que forma colonias grandes en el noroeste de Costa Rica podría ser la misma especie que se encuentra en el sur de México, pero requiere más estudio, y no son nada claros los nombres correctos para los miembros de este complejo (salvo *S. labiata* Warsz. & Rchb.f. y *S. warszewiczii*). Más al sur en Costa Rica parece que hay varias especies distintas y algunos enjambres híbridos.

ABSTRACT. *Sobralia warszewiczii* Rchb.f. Rchb.f. was described from western Panama in 1852. The name has been used as far away as southern Mexico, though, in fact, it is not definitely known from Costa Rica, where there are several species with lilac or purple flowers. A species that forms large colonies in northwestern Costa Rica may well be the same species that occurs in southern Mexico, but more study is needed, and it is not clear what the correct names are for any of this complex (except for *S. labiata* Warsz. & Rchb.f. and *S. warszewiczii*). Farther south in Costa Rica, there appear to be several species and what are apparently hybrid swarms.

PALABRAS CLAVE / KEY WORDS: Orchidaceae, clasificación, classification, *Sobralia*, *S. warszewiczii*, enjambres híbridos, hybrid swarms

En las últimas décadas, algunos colegas y su servidor hemos descrito aproximadamente 20 especies de *Sobralia*. Ahora tenemos algunas otras en preparación, y media docena que se pueden publicar tan pronto que haya material suficiente. Aquí, al contrario, quiero discutir algunas poblaciones a las cuales prefiero no aplicar nombres, por lo menos sin mucho más estudio. Las flores de *Sobralia* son sumamente delicadas, por lo cual es muy difícil identificar material prensado. A veces puede ser útil visitar las localidades típicas, donde los holotipos se colectaron, para ver si uno puede encontrar plantas que concuerden bien con las descripciones originales.

Las primeras especies de *Sobralia* de Costa Rica y Panamá fueron descritas por Reichenbach en 1852. La mayoría de ellas son nítidas y fáciles de reconocer, con la excepción notoria de *S. bletiae* Rchb.f. El tipo de *S. bletiae* tiene un pétalo ligeramente adherido al labelo en la base (afortunadamente, este holotipo se conserva muy bien). Reichenbach interpretó esto como un lóbulo lateral, y en su dibujo agregó otro igual al

otro lado. Cuando vió la misma especie de nuevo, la describió como *S. suaveolens* Rchb.f.

Sobralia warszewiczii también fue descrita en 1852, y el nombre se ha usado desde Panamá hasta el sur de México. El Herbario Reichenbach contiene una muestra prensada más bien fea, probablemente preparada por Warszewicz en el campo. En 1866, Reichenbach publicó un dibujo de la flor, pero el dibujo muestra verrugas prominentes que no concuerdan muy bien con el ejemplar prensado. En esa ocasión Reichenbach escribió (en alemán) “Solamente en lugares húmedos en el Volcán Chiriquí” [ahora V. Barú], y “morada brillante.” Afortunadamente, hemos encontrado plantas grandes de flores morado brillante a aproximadamente 2000 m de altura en el Volcán Barú, y confiamos que esta es la auténtica *Sobralia warszewiczii*. Hasta ahora, no hemos encontrado plantas de *S. warszewiczii* en Costa Rica, pero es muy probable que aparezcan, porque una de las poblaciones está muy cerca de la frontera tica.

¿Qué otras especies de flor lila hay en Costa Rica?

Aparte de *S. labiata* que es muy distinta, dos supuestas especies se han descrito. *Sobralia amparoae* Schltr. fue colectada en el jardín de doña Amparo de Zeledón, que aparentemente estuvo hacia el lado norte de San José, pero no se sabe nada con respecto al origen de la planta. En el mismo artículo, Schlechter publicó *Sobralia bradeorum* Schltr., de “Costa Rica, San Jose, 1130 m, 1909.” Es posible que aún hubieran sobralias silvestres en San José en 1909, o aún en 1923, cuando Schlechter publicó la especie. Ahora, las esperanzas de encontrar *Sobralia* silvestre en San José son aproximadamente iguales a las que hay de encontrar *Cypripedium* silvestre en Manhattan. Además, Schlechter notó que casi todas las flores de *S. bradeorum* fueron dañadas por pequeños insectos. Hay sobralias lilas de Panamá hasta Guatemala y el sur de México, pero parece que hay varias especies distintas, por lo menos en Costa Rica y Panamá.

Según Schlechter, el labelo de *S. amparoae* tenía cinco quillas, mientras que *S. bradeorum* tenía solamente tres. Con respecto a las quillas, el complejo de *S. amparoae*, *S. bradeorum* y *S. warszewiczii*, por lo general, tienen 3, 4 o 5 quillas principales, más una quilla más baja a cada lado. Me parece que hay variación dentro de las poblaciones. Por cierto, estas son las poblaciones que podrían representar enjambres híbridos. Otra característica a la que se ha dado mucha importancia es que plantas que crecen a mayor altura o en lugares muy expuestos tienen las hojas más bien coriáceas y fuertemente acanaladas, pero si se trasplanta una de estas plantas en un invernadero, estas características desaparecen.

En la provincia de Guanacaste hay poblaciones grandes de una *Sobralia* que demuestra mucha variación en color. Hay flores muy pálidas, otras con varios tonos de lila y algunas flores blancas, todas muy parecidas en estructura. Las poblaciones grandes de Guanacaste no muestran floración gregaria, sino que hay algunas pocas plantas en flor casi todos los días (en la época de floración). Las plantas de Guanacaste muestran el mismo comportamiento en cultivo. Más al sur, en la provincia de Alajuela, hay plantas más dispersas que parecen tener floración gregaria, pero la muestra aún es muy limitada. Al parecer, las poblaciones grandes de Guanacaste logran la polinización sin floración gregaria. De lo que he visto, las plantas de Guatemala

y Chiapas bien podrían ser la misma especie que forma poblaciones grandes en Guanacaste, pero, otra vez, no hay aún una muestra adecuada.

En este momento, me parece que hay varias poblaciones de flor más o menos lila en Costa Rica (además de *S. labiata*). 1. Hay una especie que se extiende desde Coto Brus hasta el Valle de Orosí, en Cartago. Suponiendo que la planta de doña Amparo fue traída por su hermano de Turrialba, es muy posible que *S. amparoae* sería el nombre correcto para esta especie (Fig. 4). 2. Por la carretera al sur de Cartago, cerca de Cangreja (km. 30- 35) hay una *Sobralia* de flor más oscura, con algo de amarillo en el centro (Fig. 2). Uno ve las flores lila oscuro de vez en cuando por la carretera, pero muchas veces las flores están muy altas e inaccesibles. Otras veces, las condiciones del tránsito no permiten una parada rápida. Hemos colectado varias plantas sin flores, pero casi siempre resultan ser otras especies muy diferentes.

El año pasado, un amigo me llevó al Alto de Araya, a apenas unos 10 km al sureste del Jardín Botánico Lankester, pero hay que bajar al Valle de Orosí y subir por una de las peores carreteras que he visto en mi vida. En el Alto hay una laguna relativamente pequeña, pero llena de sobralias creciendo en los árboles y arbustos de la laguna. La variación en la laguna es tan grande que sugiere un enjambre híbrido, tal vez entre plantas de *S. amparoae* y otras muy oscuras con el centro pálido (Fig. 5). Don Abel Araya me dice que antes habían más plantas de flor oscura, pero son muy llamativas y al parecer los visitantes se han llevado casi todas las plantas de la laguna. Don Abel amablemente nos regaló una división de la planta en su jardín y está creciendo muy bien (ya tenemos plántulas en frasco.) Sería muy interesante hacer un estudio detallado de la población del Alto de Araya, pero la condición de la carretera no ayuda mucho. También hay lo que parece ser otro enjambre híbrido en La Laja, más al sur, también en Orosí. Afortunadamente, La Laja es de más fácil acceso, y creo que será mucho más factible hacer un estudio detallado con las plantas de La Laja. Las sobralias grandes, incluyendo las de flor lila, frecuentemente forman colonias grandes a los lados de carreteras nuevas, y en otros declives empinados y perturbados. Dodson (1998) sugirió que las especies *S. ecuadorana* Dodson, *S. gentryi* Dodson y *S. powellii*



FIGURE 1. *Sobralia warszewiczii*, una planta recolectada en el area de Río Sereno, Panamá, cerca de la frontera con Costa Rica, pero aún no hemos encontrado la especie en Costa Rica.



FIGURE 2. *Sobralia* sp., Esta especie se encuentra cerca de Cangreja, al sur de Cartago, Costa Rica.



FIGURE 3. *Sobralia* sp. Alto de Araya (Cartago, Costa Rica); una división de la planta que crece en el jardín de don Abel Araya. Podría ser la única planta de esta especie que aún se encuentra cerca de la laguna.



FIGURE 4. ¿*Sobralia amparoae*? Esta especie se encuentra de Coto Brus (Puntarenas, Costa Rica) hasta el área de Tapantí (Cartago, Costa Rica). Parece ser un elemento en los enjambres híbridos de La Laja y Alto de Araya. Aún no es claro cuales otras especies contribuyan genes a los enjambres híbridos.



FIGURE 5. Una muestra de flores recolectadas por don Abel Araya en la laguna, Alto de Araya (Cartago, Costa Rica). La variación sugiere un enjambre híbrido.

Schltr. forman un enjambre híbrido a los lados de las carreteras, aunque las mismas especies no parecen formar híbridos en regiones no alteradas. Es muy probable que la “*S. powellii*” del Ecuador no tenga nada que ver con la que fue descrita de Panamá central, y hasta es posible que la “*S. powellii*” ecuatoriana sea una “especie” de origen híbrido.

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FILOGENIA MOLECULAR PRELIMINAR DE *SCAPHOSEPALUM* (ORCHIDACEAE: PLEUROTHALLIDINAE)

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RESUMEN. El género *Scaphosepalum* (Orchidaceae: Pleurothallidinae) agrupa a 49 especies de distribución tanto amplia como restringida las que muchas veces crecen en simpatria biótica en los bosques montanos neotropicales. *Scaphosepalum* alcanza su pico de diversidad en el norte de los Andes y constituye un sistema interesante para investigar el efecto que tuvo el levantamiento de los Andes en los procesos de especiación. El objetivo de este proyecto es crear un marco filogenético que sea la base para evaluar si la distribución actual de las especies es la consecuencia de especiación simpátrica o especiación alopatrica seguida por contacto secundario. A continuación se presenta la filogenia preliminar del género *Scaphosepalum* reconstruida utilizando caracteres moleculares obtenidos de la amplificación y secuenciación de las regiones ITS, *trnL-F*, *matK*, y *ycf1*. Los datos de las cuatro regiones combinadas fueron analizados bajo los criterios de máxima parsimonia (MP), máxima verosimilitud (Maximum Likelihood: ML) y bayesiano y obtuvieron reconstrucciones filogenéticas similares. A pesar de que esta reconstrucción filogenética está aún en una etapa preliminar, los resultados sugieren una fuerte estructura geográfica.

ABSTRACT. The orchid genus *Scaphosepalum* (Orchidaceae: Pleurothallidinae) encompasses 49 species widely or narrowly distributed in biotic sympatry in the montane Neotropical forests, where it reaches its peak of diversity. *Scaphosepalum* represents an interesting system to investigate speciation patterns. The main goal of this project is to reconstruct the evolutionary history of *Scaphosepalum* and use the resulting phylogenetic hypothesis to determine if the current sympatric distribution of the species is the result of sympatric or allopatric speciation followed by secondary contact. The preliminary phylogeny presented here is based on novel molecular data obtained from the amplification and sequencing of the combined gene regions ITS, *trnL-F*, *matK*, and *ycf1*. Analyses were performed using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian optimization criteria. All the resulting analyses resulted in similar tree topologies and indicate a strong geographical structure in the dataset.

PALABRAS CLAVE / KEY WORDS: Orchidaceae, *Scaphosepalum*, filogenia molecular, molecular phylogeny

Scaphosepalum agrupa a 49 especies y es un clado relativamente pequeño que pertenece a la diversa subtribu Neotropical Pleurothallidinae la que cuenta con aproximadamente 4000 especies (Pridgeon *et al.*, 2005). *Scaphosepalum* puede ser fácilmente reconocido por sus flores no resupinadas y sinsépalos coronados por osmóforos (Fig. 1), los que en la mayoría de las especies, están bien desarrollados (Vogel, 1965; Pridgeon & Stern, 1985; Luer, 1986, 1988; Pridgeon *et al.*, 2005). Los sinsépalos y el sépalo dorsal poseen caudas (colas sepalinas), las cuales han sido motivo de confusión taxonómica y la razón por la que

inicialmente se incluyó a este género en *Masdevallia* (Luer, 1986).

El pico de diversidad del género *Scaphosepalum* y de la subtribu Pleurothallidinae se encuentra en los bosques montanos del norte de los Andes, un paisaje que ha ofrecido múltiples oportunidades para eventos de radiación y diversificación (Gentry, 1982; Burnham & Graham, 1999; Young *et al.*, 2002; Hughes & Eastwood, 2006; Rull, 2008; Antonelli *et al.*, 2009; Graham, 2009; Struwe *et al.*, 2009). El objetivo principal de este estudio es reconstruir la filogenia de *Scaphosepalum* para examinar los patrones de especiación del género con el



FIGURA 1. Diversidad floral de *Scaphosepalum*. A. *S. decorum*; B. Flor tubular de *S. odontochilum*; C. *S. medinae*. *osm*: osmóforos, *ds*: sépalo.

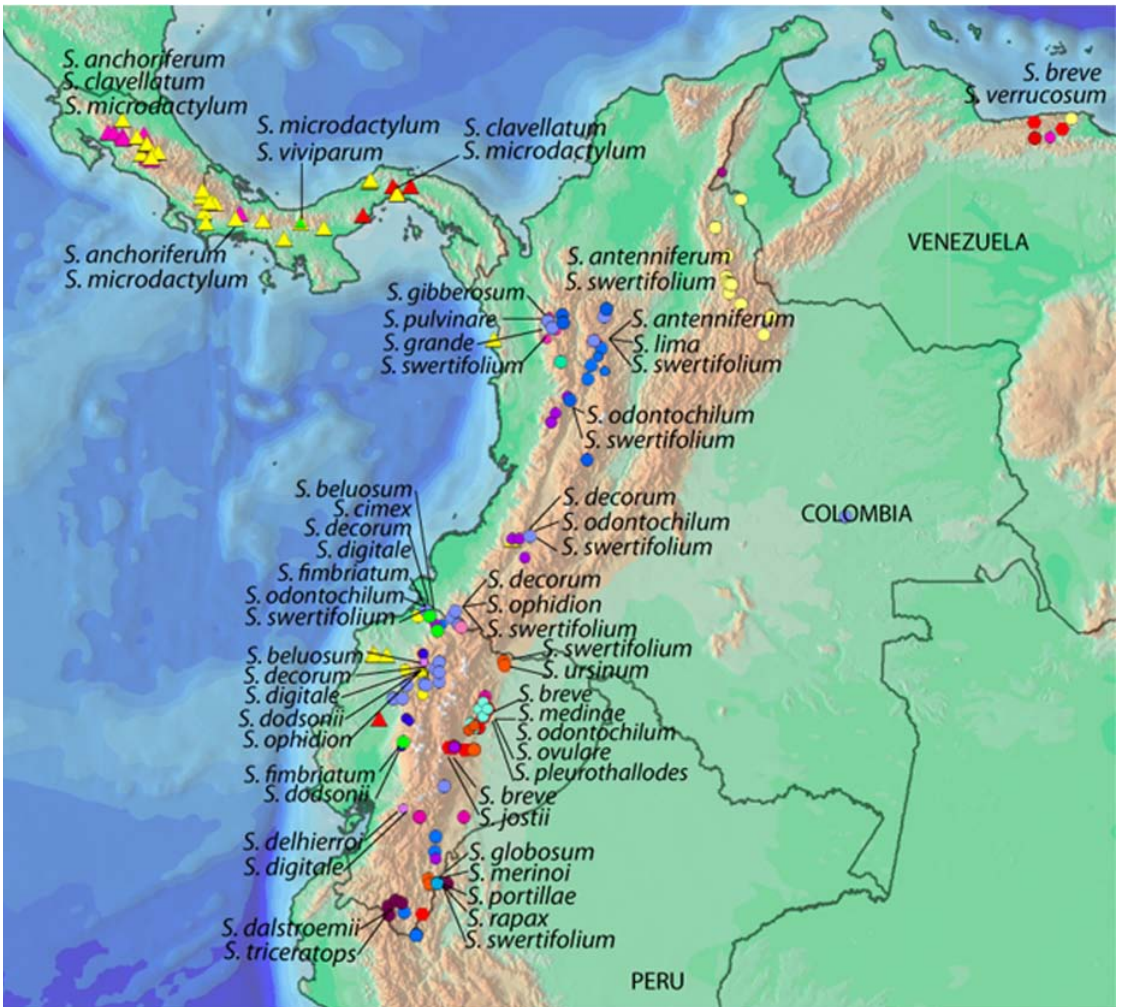


FIGURA 2. Distribución de *Scaphosepalum* y las localidades en donde varias especies crecen en simpatría biótica. Las localidades conocidas en el escudo Guyanés, Bolivia, y Perú fueron excluidas de esta figura.

TABLA 1. Cebadores (primers) utilizados en la amplificación y secuenciación de regiones nuclear y plástidos del género *Scaphosepalum*.

Cebadores	Gen nuclear	Genes plástidos		
	ITS	<i>matK</i>	<i>trnL-F</i>	<i>ycf1</i>
Amplimero	ACGATTTTCATGG	<u>-19F</u>	<u>C</u>	<u>3720F</u>
	TCCGGTGAAGT	CGTTCTGACCATATT	CGAAATCGGTAGA	TACGTATGTAATGAAC
	GTTTCG	GCACTATG	CGCTACG	GAATGG
Amplimero inverso	TAGAATTCCCCG	<u>trk2r</u>	<u>F</u>	<u>5500R</u>
	GTTGCTCGCCG	ACCTAGTCGGATGG	ATTTGAAGTGGTGA	GCTGTTATTGGCATCA
	TTAC	AGTAG	CACGAG	AACCAATAGCG
Interno		<u>int F</u>	<u>D</u>	<u>560F</u>
		TGAGCGAACACATT	GGGGATAGAGGGA	GATCTGGACCAA
		TCTATGG	CTTGAAC	TGCACATATT
Interno inverso		<u>int R</u>	<u>E</u>	<u>850R</u>
		ATAAGGTTGAAACC	GGTTCAAGTCCCTC	TTTGATTGGGAT
		AAAAGTG	TATCCC	GATCCAAGG

fin de comprender mejor los procesos evolucionarios que han convertido a la región Andina en uno de los puntos calientes (hot-spots) del planeta (Myers *et al.*, 2000). *Scaphosepalum* representa un sistema ideal para detectar escenarios de especiación alopátrica, parapátrica y simpátrica pues una gran cantidad de sus especies viven en simpatria (Fig. 2).

Materiales y métodos

Muestreo — Se extrajo exitosamente el ADN de 37 especímenes que representan a 28 especies con los protocolos citados en Whitten *et al.* (2007). Todos los especímenes utilizados en el análisis preliminar fueron obtenidos de ejemplares cultivados *ex situ* en el Jardín Botánico de Atlanta (ABJ) y cuentan con especímenes depositados en el herbario de la Universidad de Florida (FLAS).

Secuenciación de genes — La baja divergencia molecular constituye el mayor reto de este proyecto sin embargo, varios genes plástidos (*trnL-F*, *matK*, y *ycf1*) y un gen nuclear (ITS) han demostrado ser informativos y útiles para nuestro estudio. Todas las amplificaciones se realizaron con el kit de reactivos y polimerasa Sigma Jumpstart *Taq* y Sigma Jumpstart

redTaq (Sigma-Aldrich, St. Louis, Missouri, USA) en reacciones de 25 μ L conformadas por 2.5 μ L de buffer, 0.5 μ L de dNTPs y cebadores, 1.0 μ L de ADN total, cantidades variables de MgCl₂ (1.5 hasta 3.0 μ L), y agua destilada.

Inicialmente se amplificaron las cuatro regiones en un segmento sin embargo, para varios taxones problemáticos las amplificaciones de *matK* y *ycf1* se realizaron en dos segmentos. Los cebadores (primers) y programas utilizados en la amplificación de los diferentes genes se detallan en las tablas 1 y 2, respectivamente. Los productos amplificados fueron colocados en placas de 96 celdas y fueron secuenciados en el Centro Interdisciplinario de Investigación de Biotecnología (ICBR) de la Universidad de Florida en Gainesville. Los electroferogramas resultantes fueron editados en el programa Sequencher 4.6 (Genecodes Inc., Ann Arbor, Michigan, USA) y alineados manualmente en el programa Se-Al (Rambaut, 2000).

Análisis filogenético — Previo a combinar las cuatro regiones moleculares y realizar los respectivos análisis filogenéticos se verificó si los sets de datos son congruentes. Para el efecto se realizaron pruebas compartimentalizadas de ILD en el programa PAUP

TABLA 2. Programas de termociclado utilizados en la amplificación de regiones nucleares y plástidas del género *Scaphosepalum*. * Programas touchdown.

	Denaturalización inicial	Denaturalización	Hibridización (Annealing)	Extensión	Extensión final	Mantenimiento
ITS*	94°C x 2 min	94°C x 1 min	76°C x 1 min	72°C x 1 min	n/a	4°C
	1 ciclo	15 ciclos				
		94°C x 1 min	59°C x 1 min	72°C x 1 min	72°C x 3 min	
		21 ciclos				
mat-K	94°C x 3 min	94°C x 30 min	50°C x 0.30 min	72°C x 2 min	72°C x 3 min	4°C
	1 ciclo	34 ciclos				
trnL-F	94°C x 3 min	94°C x 1 min	58°C x 1 min	72°C x 1:20 min	72°C x 6 min	4°C
	1 ciclo	33 ciclos				
ycf1*	94°C x 3 min	94°C x 30 min	60°C x 1 min	72°C x 2:30 min	n/a	4°C
	1 ciclo	8 ciclos				
		94°C x 3 min	50°C x 0.45 min	72°C x 2:30 min	72°C x 3 min	
		30 ciclos				

(Farris *et al.*, 1995) y pruebas de congruencia topológica para cada set de datos (Soltis *et al.*, 1998). El set de datos combinado fue analizado bajo los criterios de Máxima Parsimonia (MP), Máxima Verosimilitud (ML: Maximum Likelihood), y Bayesiano. Para los dos criterios iniciales se utilizó el programa PAUP versión 4.0 (Swofford, 1997) y para los análisis Bayesianos se utilizó MrBayes versión 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003; Altekar *et al.*, 2004). Los modelos evolucionarios que mejor explican el modo de evolución de los diferentes sets de datos fueron obtenidos a través del programa Modeltest 3.7 (Posada, 1988).

Se utilizó parsimonia Fitch no ponderada y no ordenada en el análisis parsimónico. En las búsquedas heurísticas de MP y ML se definió un número máximo de 100 árboles, adición de secuencias al azar, con 10,000 réplicas, utilizando el algoritmo de estrategia de búsqueda múltiple (TBR, siglas en inglés). Se enraizó el cladograma con secuencias de *Dryadella*, *Platystele*, *Teagueia*, y *Pleurothallis* (*Specklinia* o *Sarcinula*) *acanthodes* sugeridos como grupos hermanos de *Scaphosepalum* (Pridgeon *et al.*, 1999,

2005). El soporte para los nodos recuperados se calculó con la medida de apoyo “bootstrap” realizando 100 reiteraciones. Para el análisis Bayesiano se definió a nst=6 y rates=invgamma, se retuvo un árbol cada 100 generaciones y se corrió el análisis por seis millones de generaciones. Los análisis fueron ejecutados en el cluster de computadores de filoinformática del Departamento de Botánica de la Universidad de Florida.

Resultados

Las pruebas compartimentalizadas de ILD indicaron congruencia entre las regiones plástidas pero incongruencia significativa entre las regiones plástidas combinadas y la región nuclear. La naturaleza de la incongruencia entre los sets de datos plástidos y el set de datos nuclear fue posteriormente analizada mediante la comparación de los valores de soporte bootstrap y las topologías de los árboles consenso estricto generados mediante MP y la topología resultante de ML. La topología del árbol correspondiente a las regiones plástidas difirió de la topología de la región nuclear

TABLA 3. Estadísticas de las diferentes regiones utilizadas en el análisis de la filogenia preliminar de *Scaphosepalum*. Valores obtenidos vía Máxima Parsimonia, los modelos de sustitución de nucleótidos fueron obtenidos utilizando Modeltest. MP: Máxima Parsimonia.

	Nuclear	Plástidas			Combinada
	ITS	3' <i>ycfI</i>	<i>trnL-F</i>	<i>matK</i>	
# taxa utilizada	57	54	58	54	54
# caracteres	800	1635	1253	1464	152
# caracteres constantes	571	1307	1071	1259	4247
# caracteres informativos (MP)	115	149	74	87	405
# caracteres no informativos (MP)	114	179	108	118	500
Mejor árbol	407	534	234	274	1450
Número de árboles	26	24	168	232	22
CI	0.73	0.69	0.82	0.81	0.72
RI	0.81	0.77	0.85	0.83	0.77
# de nodos con medida de apoyo	22/41	14/39	14/25	16/32	32/36
bootstrap $\geq 70\%$					
Modelo de sustitución nucleotídica	TrN+G	TrN+G	F81+G	F81+G	TVM+I+G

(ITS) en varios nodos cerca de la espina no resuelta del árbol nuclear. Estas diferencias fueron consideradas como incongruencia suave por lo que se procedió a combinar los datos de las tres regiones plástidas y la región nuclear.

La Tabla 3 detalla las estadísticas de los árboles obtenidos en base a los análisis parsimónicos realizados para cada región y para las cuatro regiones combinadas (ITS, *trnLF*, *matK*, y *ycfI*) así también como los modelos utilizados en los análisis de ML. Las topologías de las filogenias obtenidas mediante MP, ML, y análisis Bayesianos son similares y difieren únicamente en las longitudes de las ramas. Por razones de espacio se presenta únicamente la hipótesis evolutiva resultante del análisis de ML (Fig. 3).

Discusión

Aunque en una etapa preliminar (28 especies muestreadas de 49), la filogenia generada (Fig. 3) presenta una estructura geográfica fuerte y los patrones

filogeográficos observados reafirman la importancia de los Andes como una barrera de dispersión. Dos clados están confinados a las estribaciones orientales de los Andes. Es posible observar en la Figura 3 que un clado con alto soporte bootstrap contiene prácticamente a todas las especies muestreadas de las estribaciones noroccidentales de los Andes y América Central con potenciales casos de dispersión hacia las estribaciones orientales de los Andes centrales en Ecuador. Los dos clados restantes contienen a la mayoría de las especies encontradas en las estribaciones orientales de los Andes (color amarillo en la Figura 3). Es posible inferir en base a la filogenia preliminar (Fig. 3) y la distribución de las especies que viven en simpatria (Fig. 2), que las especies que habitan en los mismos hábitats no están cercanamente relacionadas, en otras palabras, no son los grupos hermanos inmediatos, por ejemplo, *Scaphosepalum beluosum*, *S. decorum*, *S. dodsonii*, *S. digitale*, y *S. ophiodon* están presentes en los mismos hábitats (Fig. 2) sin embargo no son

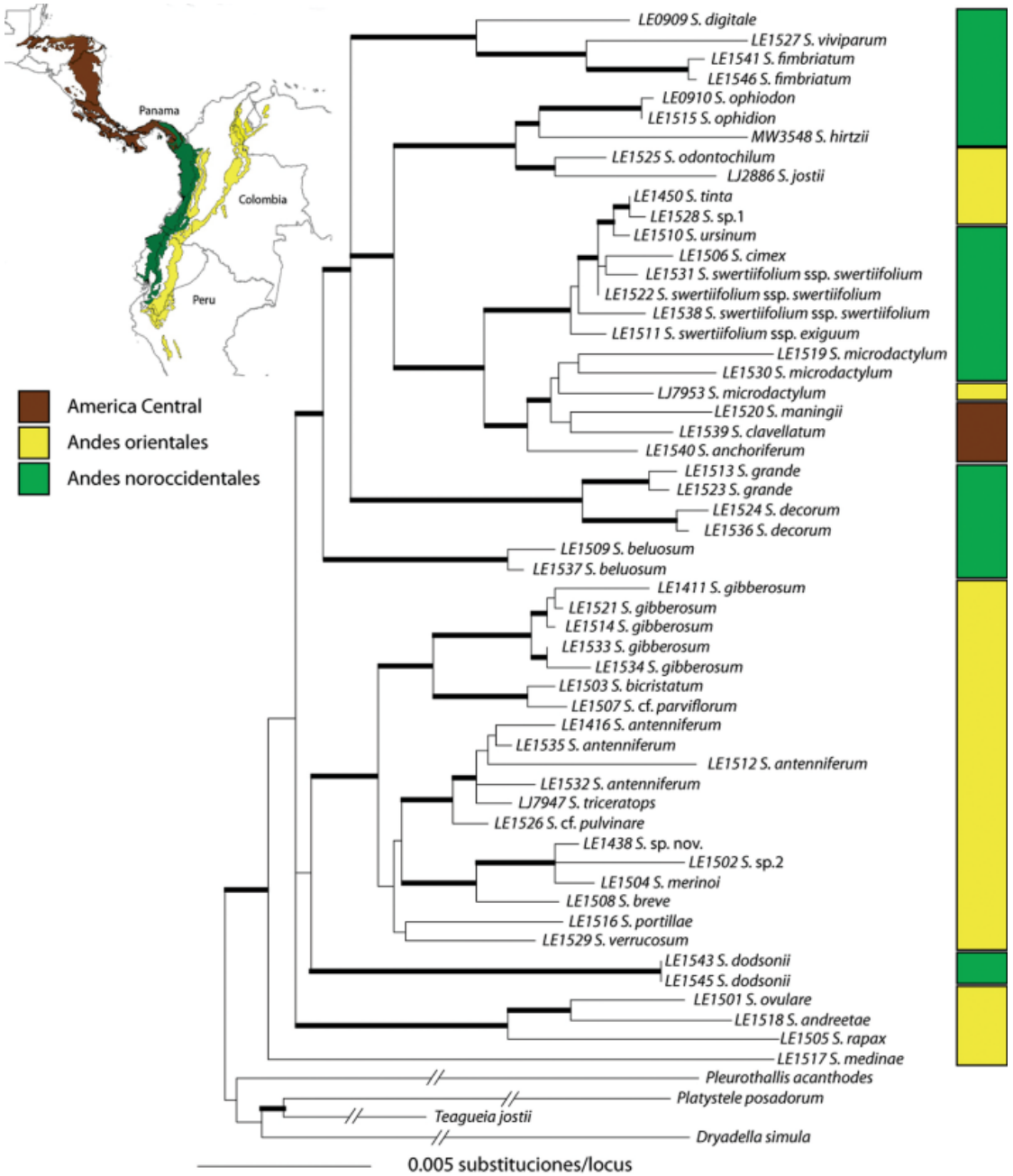


FIGURA 3. Filogenia preliminar de *Scaphosepalum*. Análisis combinado utilizando las regiones moleculares ITS, *matK*, *trnL-F*, y *ycf1* bajo el criterio de máxima verosimilitud (modelo TVM+I+G, 10,000 reiteraciones, algoritmo TBR). Las líneas engrosadas representan valores de apoyo bootstrap mayores a 75%. Las barras inclinadas representan sectores en donde las ramas han sido artificialmente reducidas para una mejor representación de la longitud de las ramas del grupo interno.

especies hermanas por lo tanto no son el resultado de especiación simpátrica.

La parafilia observada a nivel específico en *Scaphosepalum swertiifolium* (Fig. 3), una especie ampliamente distribuida es un evento común y provee evidencia sobre la divergencia de las especies por medio de un evento fundador o divergencia local (Reiseberg & Brouillet, 1994; Howard & Berlocher, 1998). A futuro se espera aumentar más especímenes y variaciones geográficas de las especies ampliamente distribuidas y también especies que exhiben una amplia variabilidad morfológica en su rango de distribución (*Scaphosepalum antenniferum*, *S. breve*, *S. microdactylum*, y *S. verrucosum*) para explorar escenarios de parafilia a nivel específico.

Llama la atención la posición de *Scaphosepalum medinae* como especie hermana al resto de *Scaphosepalum*. *Scaphosepalum medinae* (Fig. 1c) y *S. pleurothallodes*, la última, una especie no muestreada en la filogenia preliminar, son las únicas especies subresupinadas en el género con inflorescencias subcimosas. Probablemente *S. pleurothallodes* también sea hermana al resto de *Scaphosepalum*. La semejanza morfológica de *S. pleurothallodes* y *S. medinae* a *Pleurothallis* (*Specklinia* o *Sarcinula*) *acanthodes*, resulta interesante pues *P. acanthodes* crece en simpatria con las dos especies de *Scaphosepalum* antes mencionadas y podría ser el taxón hermano del clado de *Scaphosepalum*. Para comprobar la monofilia de *Scaphosepalum*, es nuestro objetivo incluir a otras especies de *Pleurothallis* (*Specklinia* o *Sarcinula*). Es también una prioridad de nuestro proyecto un muestreo completo y denso de taxones ya que las reconstrucciones filogenéticas son sensibles a las estrategias de muestreo de taxones y caracteres (Graybeal, 1998; Zwickl & Hillis 2002).

A pesar de que varias especies no están representadas en la filogenia generada, los resultados preliminares favorecen a escenarios de especiación alopátrica y parapátrica seguidos por contacto secundario de las especies.

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THE GENUS *CORYANTHES*: A PARADIGM IN ECOLOGY

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ABSTRACT. Since Darwin, the pollination biology of *Coryanthes* has fascinated naturalists, but other aspects of its biology are equally interesting. Herman Crüger, Director of the Trinidad Botanical Gardens, first described the pollination process in 1864. He talked about the strong attraction of the floral scent to some kind of Hymenopteran, but the true nature of the reward was not discovered until 100 years later by Dodson and Vogel. *Coryanthes* species grow exclusively in so-called antgardens. These arboreal communities can reach diameters of 150 cm with the ant nest comprising 80 cm. Both organisms share a destiny because the plant is condemned to death if the associated ant colony dies. The plants offer nectar in extrafloral nectaries and provide a framework for nest construction with their root system, while the ants defend the plants against herbivores and additionally fertilize them with vertebrate feces. This abundant provision of nutrients by the ants allows the plants to grow rapidly. *Coryanthes* plants raised artificially from seed require about 2 to 3 years to flower. This is perhaps one of the most rapid maturation times among orchids and is more remarkable because the flowers of *Coryanthes* species are large and massive. They can reach a weight of more than 100 g, the most massive within the orchid family. Capsule ripening is also rapid; most species require only two months to mature a capsule containing around 600,000 seeds. This rapid maturation probably represents an adaptation to this vulnerable habitat. The pollination process is finely tuned with the attraction of one or a few species of male euglossine bees; no other type of insect can effect pollination. A bee that is too large for the passage between the epichile and column will die because it cannot escape the flower and seals the exit. A bee that is too small might pass without effecting pollination. This attraction of one or a few species of bee is the result of the specific preferences of the euglossine males to the chemical composition of the floral fragrance. The different species of *Coryanthes* have different scents, helping the taxonomist to delimit species that in nature are pollinated by the same bee in spite of color or form variations. All these peculiar ecological adaptations of *Coryanthes* are evolutionary solutions to survive extreme challenges. The ecology of these plants is so multifaceted that even after years of intense investigation they still hide some secrets.

RESUMEN. Desde la época de Darwin, la biología de polinización de *Coryanthes* fascinó a los naturalistas, pero no solo con este fenómeno este género sorprende a sus observadores. Dr. Crüger, el director del Jardín Botánico de Trinidad fue el primero que investigó el proceso de la polinización en 1864. Él habló de la intensa atracción del aroma floral a ciertos himenopteros, pero la verdadera recompensa buscada por las abejas fue descubierta hace tan sólo 100 años por Dodson y Vogel. Todas las especies de *Coryanthes* crecen únicamente en llamados jardines de hormigas, éstas comunidades pueden llegar a un tamaño de 150 cm de diámetro, solo el nido de las hormigas mide unos 80 cm. Los dos organismos son dependientes uno del otro, la planta esta condenada a la muerte si la colonia de hormigas asociada muere. Las plantas ofrecen nectar en nectarios extraflorales y con su sistema de raíces facilitan una base para la construcción del nido, mientras las hormigas defienden su planta contra herbívoros y las fertilizan con los excrementos de vertebrados colectados del suelo. La alimentación abundante permite a la planta un crecimiento muy rápido. Plantas de *Coryanthes* que crecen en viveros necesitan nada mas entre 2 a 3 años para florecer. Esto es tal vez es uno de los record en las orquídeas, porque las flores son muy grandes y pesadas. Estas pueden llegar a un peso de mas que 100 g, el cual es otro record dentro de la familia de las orquídeas. Las semillas para madurar necesitan solo 60 días, una cápsula contiene alrededor de 600.000 granos de semilla, eso probablemente es una adaptación a su habitat tan vulnerable. El proceso de polinización es sincronizado con gran precision para atraer a una o pocas

especies de euglosidos, solamente ellos pueden efectuar la polinización. Si una abeja demasiado grande para el pasaje de la flor es atraída, ella tapa la salida de la orquídea, resultando en la muerte de ambas. Una abeja demasiado pequeña puede pasar la flor sin efectuar la polinización. La atracción individualizada esta causada por la preferencia específica de los machos de euglosidos en la composición química de los aromas florales. Las diferentes especies adentro del género *Coryanthes* tienen diferentes aromas florales, ayudando así también al taxónomo para distinguirlas o en otro caso unir especies variables cuales clones están polinizados por la misma especie de abeja. Todas estas peculiaridades de las especies de *Coryanthes* mencionadas aquí son las respuestas de la evolución a la sobrevivencia en una ecología extrema. La ecología de estas plantas es tan compleja y diversificada que a pesar de un largo plazo de investigación intensiva, todavía no se conoce a cabalidad ó en totalidad una serie de procesos.

KEY WORDS: Orchidaceae, *Coryanthes*, ecology, pollination

From the beginning of cultivation of tropical orchids, plants of *Coryanthes* were in great demand by horticulturalists. This was mainly because of their bizarre flower morphology and the great difficulties in keeping them alive for more than a single flowering season. Even skilled growers found the cultivation of these plants a hard nut to crack. From the beginning of the 1970s some gardeners developed successful methods of cultivation for these fascinating plants. My story of *Coryanthes* and their biology started 30 years ago in the Botanical Garden of Heidelberg with my colleague, the famous orchid gardener Hans Gerhard Seeger. Together we were the first to assemble more than 30 species in the hot and humid greenhouse, and with our success the idea evolved to monograph the genus. I remember well when Cal Dodson encouraged me to work on this genus, because no one in the Neotropics could grow *Coryanthes* well for any length of time.

The genus *Coryanthes* was established by Hooker in 1831 and published in *Curtis's Botanical Magazine* together with a colored plate. Sixty-two names belonging to this genus have been published, but only 41 species are regarded here as valid. Only 14 valid species were published by different authors before 1980, so 27 species were described within the last 30 years. The scarcity of *Coryanthes* in herbaria has several possible explanations: 1) Plants are not frequent in the wild; 2) they are well protected by stinging ants; and 3) the flowers last for only two or three days. These factors result in infrequent collection of *Coryanthes* by botanists.

Plants of *Coryanthes* grow in humid lowland forests from sea level up to elevations of 1500 m. They

are frequently found along rivers and creeks; these habitats could reflect humid microclimates or they may simply represent a collection artefact caused by the easy accessibility from a dugout, the easiest way to travel in Amazonian lowland forest. Their geographical distribution ranges from Mexico (Veracruz, *Coryanthes picturata* Rchb.f.) to Bolivia (Cochabamba, *Coryanthes vasquezii* Dodson, *Coryanthes macrantha* Hook.), and Brazil (Espírito Santo, *Coryanthes speciosa* Hook. var. *espiritasantense* Ruschi).

Coryanthes species grow epiphytically in so-called ant-gardens (Fig. 1), which are the most complex and sophisticated of all mutualistic partnerships between ants and flowering plants. These gardens consist of masses of soil, detritus, and chewed plant parts assembled at the branches of trees, forming huge clumps that may reach diameters of around 80-100 cm. All plant members in these partnerships are highly adapted, most of them obligate ant-garden plants. They are found in different plant families, e.g. Gesneriaceae (*Codonanthe*), Araceae (*Anthurium*), Cactaceae (*Epiphyllum*), Bromeliaceae (*Aechmea*), Piperaceae (*Peperomia*), Orchidaceae (*Coryanthes*, *Epidendrum imatophyllum* - obligate; *Gongora*, *Sievekingia* - facultative). Most of them bear seeds with elaiosomes or arils that induce the ants to collect the seeds and place them in their nests. As the plants grow, nourished by the soil and other materials, their roots become part of the framework of the gardens. The ants in turn feed on the nectar offered in extrafloral nectaries and fruit pulp provided by the plants. The garden is strictly controlled by the ants; legitimate members are tended by the ants, but invaders to this favorable substrate are pruned by them.



FIGURE 1. Ant-garden with *Coryanthes flava*, mangrove swamps of Mecana, Dept. Chocó, Colombia. The association consists of *Epidendrum*, *Epiphyllum* (Cactaceae), *Peperomia* (Piperaceae), *Anthurium* (Araceae), and some Bromeliaceae. (Photo: G. Gerlach)

In *Coryanthes* ant-gardens, the ants belong mainly to the genera *Crematogaster*, *Azteca* (Myrmicinae), and *Camponotus* (Dolichoderinae). The *Coryanthes* plant quickly develops an extensive root system that serves as a matrix for nest construction. Erect roots ('trash-basket roots') are often present; they collect leaf litter that decomposes around the plant, providing a constant flux of nutrients for the plant. Extrafloral nectaries occur on the plants' most vulnerable parts: new shoots, bracts, and the outer surfaces of the sepals when the flower still is in bud (Plate 1). The ants defend their sugar source against herbivores, maintain their garden, and fertilize their partners with vertebrate feces collected from the ground. Because of attentive cultivation by ants, *Coryanthes* species are rapid growers. If well provided with nutrients and the

other requirements, an artificially raised *Coryanthes* can flower within 2 1/2 years after sowing, a record within Orchidaceae keeping in mind that the flowers of this genus hold another record as the heaviest among orchids. The question in this mutualistic relationship is whether a *Coryanthes* seedling first colonizes an existing ant nest or the *Coryanthes* first establishes a root system that is subsequently colonized by the ants. Long internodes and small, scale-like leaves of seedlings sown asymbiotically in flasks support the hypothesis that the *Coryanthes* seed germinates deep in an ant nest and that the young seedling with its long internodes reaches the surface of the nest and then change its habit, developing the short internodes characteristic of the adult stage (Meyers and Lamb, 2009). Horich (personal communication) observed ants collecting seeds of an *Epidendrum* species growing in ant nests in Costa Rica. Dodson (cited by Benzing, 1984) argued that lipid deposits within *Coryanthes* seeds may also be attractive to ants, but to date this observation has not been confirmed nor refuted. So germination in the ant nest could be explainable but still needs to be investigated and confirmed. On the other hand, it is hard to imagine that the wind-dispersed balloon-seeds liberated by *Schleuderhaare* (hairs in the capsule that help to disperse seeds when the capsule opens) are collected and dispersed by ants. Has a second dispersal mechanism evolved? It is interesting to note that often trees carry more than one ant-garden with *Coryanthes* plants. In Guatopo National Park in Venezuela, we (G. Bergold, T. Graf, and I) found an huge mango (*Mangifera indica* L., Anacardiaceae) tree with more than 10 *Coryanthes* ant-gardens. How can the fact that all the ant nests were colonized by *Coryanthes* plants be explained? Many ants live in polygynous colonies or have extensions of their primary colony. So it is easy to imagine that ants collect *Coryanthes* seeds from a capsule opening in one of their ant-gardens and carry them to their extensions. Experiments to prove this hypothesis have not been undertaken; it is difficult to maintain a living ant-garden in cultivation. Even intact ant-gardens have not survived being moved, so these experiments have to be made in nature under difficult circumstances.

The genus *Coryanthes* is divided into two sections, *C.* sect. *Coryanthes* (previously *C.* sect. *Eucoryanthes*) with a smooth mesochile and *C.* sect.

PLATE 1. Extrafloral nectaries of *Coryanthes rutkisii* Foldats, indicated by arrows. Note that they are present on median vein of lateral sepals and median vein of bract. (Photos: G. Gerlach)



Lamellunguis with a mesochile bearing warts or lamellae. Micromorphology of the seeds (Plate 3) differs significantly between the sections. The seeds are all elongate, but those of *C.* sect. *Coryanthes* are fusiform, whereas those of *C.* sect. *Lamellunguis* have only one tapered side. Seed ripening is remarkably quick in *Coryanthes* species; most capsules need only 60 days to develop (Gerlach, 1993; Babczinsky *et al.*, 2009). Similarly short times for seed ripening elsewhere in Stanhopeinae are found only in some species of *Gongora*, which occupy similar habitats. The other genera within this subtribe need more than six months to produce ripe seeds.

Molecular phylogenetic studies utilizing nrITS have not provided much resolution. However, they do support division into these two sections (Fig. 2), as well as a third clade composed of species with a nearly flat hypochile (versus semiglobose, cup-shaped), including the isolated species *C. macrocorys* Rolfe, the phylogenetic relationships of which were previously unclear. Erection of additional sections within the

genus should await additional data that provide better resolution and support.

Coryanthes possess trap-flowers, with a spatial separation of anther and stigma that prevents self-pollination. Most non-orchid trap-flowers are proterogynous. In *Aristolochia* L. (Aristolochiaceae) species, for example, the flowers are first in the female phase, in which the pollinator enters the trap and leaves its pollen load at the stigma and cannot escape. On the following day the flowers switch to the male phase, releasing pollen onto the pollinator; the male and female phases are separated by time. Finally, the trap opens by wilting of the hairs that previously impeded the exit of the pollinators. In *Coryanthes*, however, the pollinator is forced to pass first by the stigma and then the anther; the separation of the sexes is here by space. From artificial pollinations we know that the stigma is receptive as soon as the flower opens.

Looking at the micromorphology of these trap-flowers, one can detect several adaptations to keep the pollinators from escaping by flying or crawling out

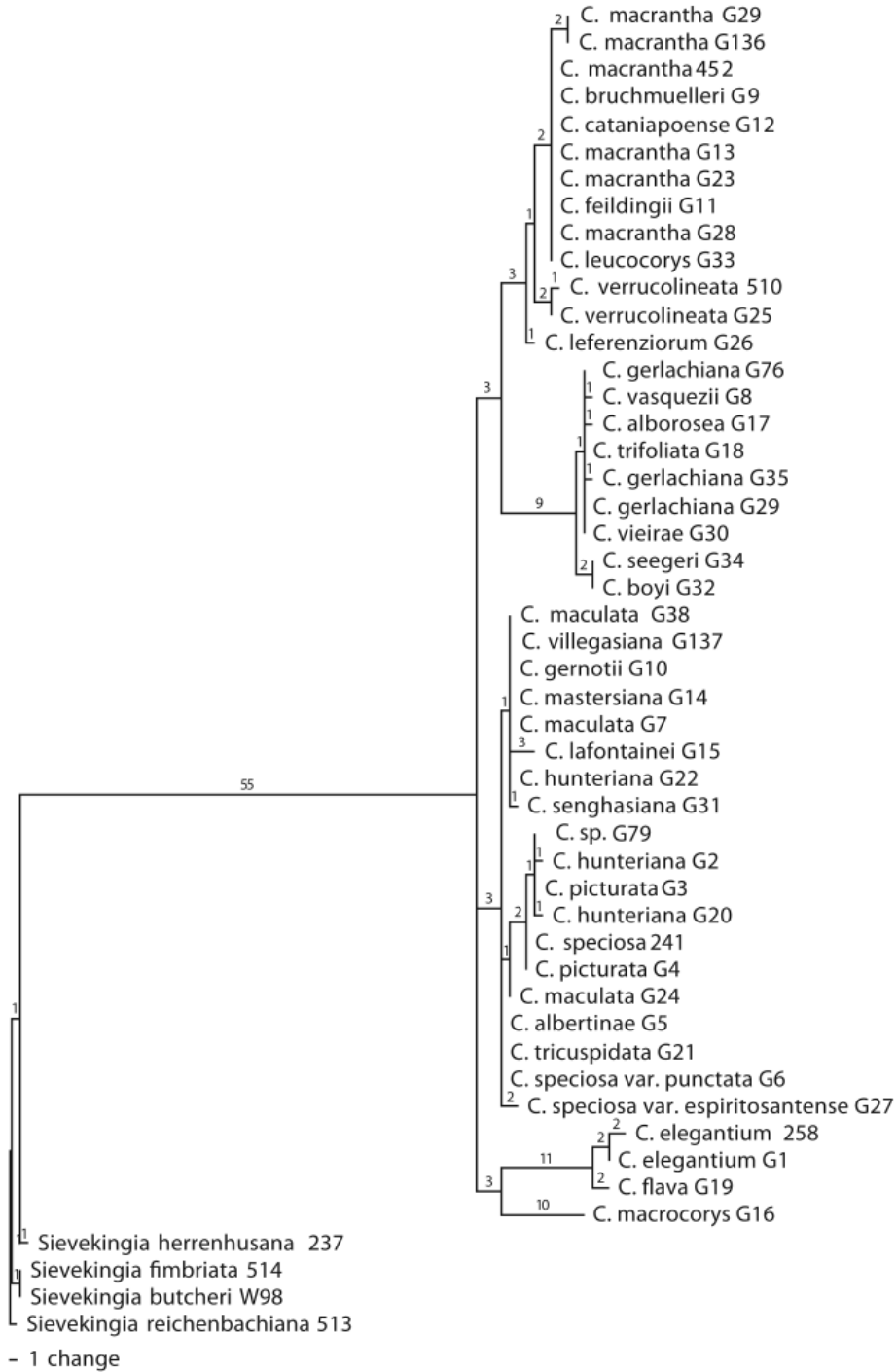


FIGURE 2. A single most-parsimonious tree from cladistic analyses of nrITS sequences of *Coryanthes* (Whitten and Gerlach, unpublished). Values above branches are number of steps.

PLATE 2. SEM of surfaces of the flower of *Coryanthes speciosa* var. *espiritosantense*. (Photos: G. Gerlach).

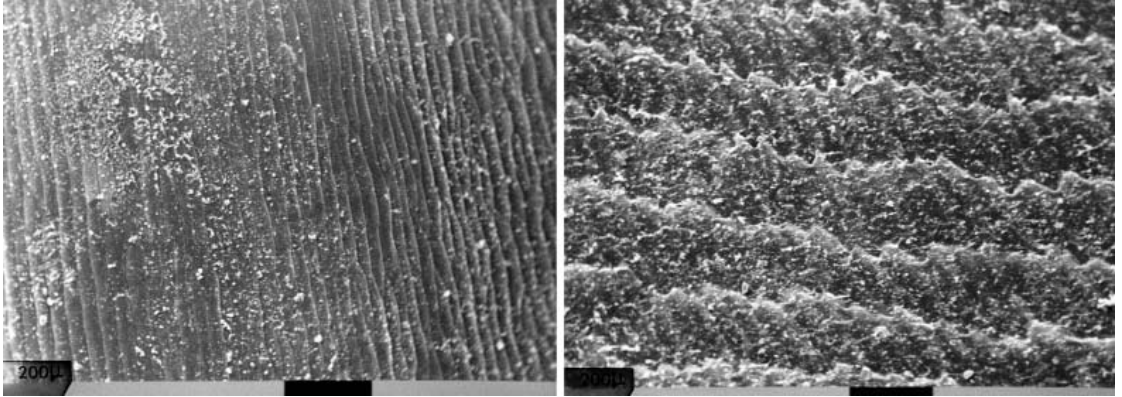
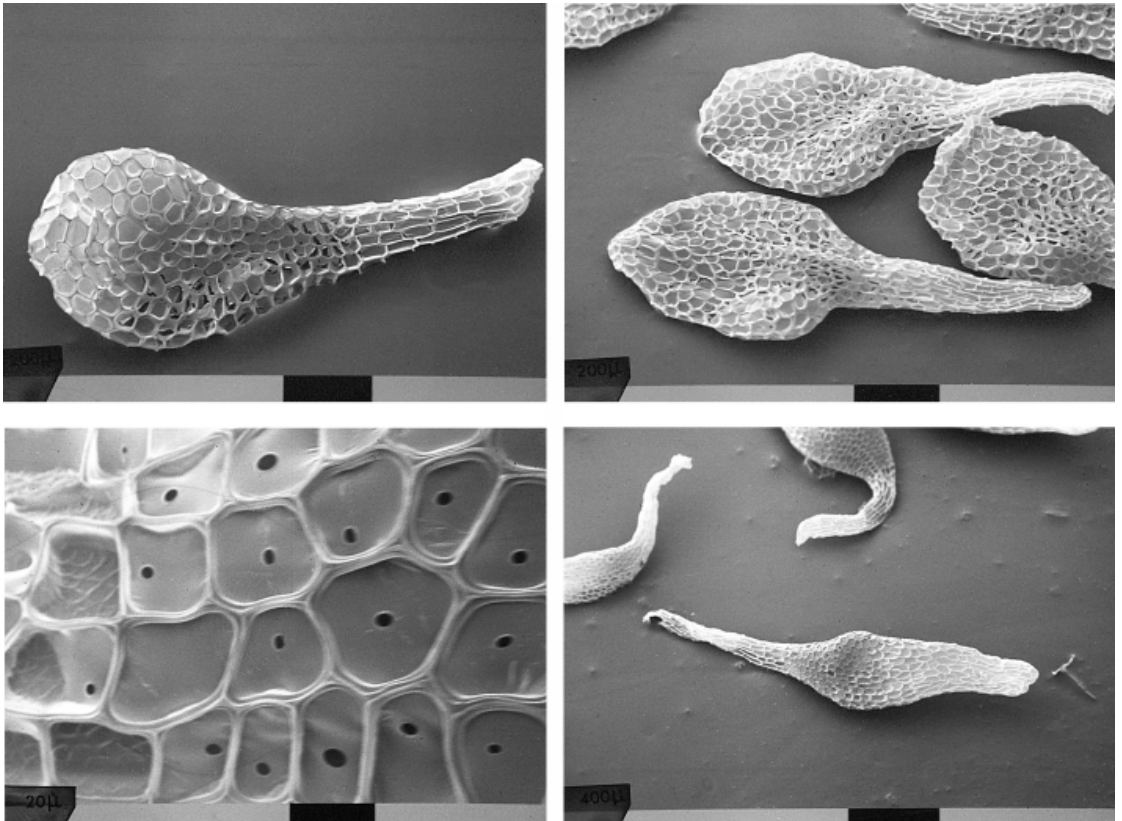


PLATE 3. SEM of the seeds of *Coryanthes*. From left to right, upper row — *C.* sect. *Lamellunguis*: *C. rutkissii* Foldats, *C. trifoliata*; lower row — *C.* sect. *Coryanthes*: *C. gernetii* G.Gerlach & Romero, *C. albertinae* (Photos: G. Gerlach).



of the bucket (epichile). The pollinator is prevented from flying out of the bucket because its wings are wet from the liquid dripping from the basal glands and stored in the bucket. The liquid is not pure water (nor nectar) but contains some soap-like substance (saponins?) that disrupt surface tension of the liquid.

The walls of the bucket formed by the lip show cell arrangements similar to the carnivorous pitcher plants *Cephalotus follicularis* Labill. (Cephalotaceae) and *Darlingtonia californica* Torr. (Sarraceniaceae), which are not related to orchids (Barthlott and Ehler, 1977). The cells overlap a bit like roof tiles, and their surfaces

PLATE 4. Pollination of *Coryanthes kaiseriana* by *Euglossa alleni* in Costa Rica. From left to right: pollinator encompasses the midlobe of the epichile, trying to use his foreleg to pull himself out of the flower; pollinator nearly ready to escape the flower, the pollinarium now fixed on his body and the anther cap just before falling off; bee in recovery phase, in the drying process before leaving the flower. (Photos: G. Gerlach)



are covered by epicuticular waxes (Plate 2). These waxy, shingle-like surfaces are slippery, preventing the bees from climbing out of the bucket. The column also forms part of the trap; the cells lie side by side and also are covered by waxes.

Like all other Stanhopeinae, flowers of *Coryanthes* are pollinated by male, perfume-collecting euglossine bees. The floral fragrance is produced by osmophores hidden under the mostly cup-shaped hypochile of the lip. These volatile substances are both the attractant and reward for the bees. Each bee species has species-specific fragrance preferences that may also vary geographically and seasonally. In addition to orchid flowers, male euglossines collect volatiles from rotten wood or feces.

After collecting the fragrance compounds at the horn-shaped osmophore below the hood (hypochile) of the lip using its feathery, foretarsal brushes, the male euglossine bee hovers and transfers the accumulated chemicals to its hollow, inflated hind tibiae. Flying around with other individuals, eventually competing

with them, the pollinator comes into contact with drops on the pleuridia (liquid-secreting glands at the base of the column) and falls in the liquid-filled bucket (epichile). From here the bee cannot escape because of the smooth surface of the inner side and its now-moistened wings. A lip callus at the level of the liquid provides a foothold and directs the bee towards the exit passageway formed by the tip of the lip and the column apex. The claw of the lip is flexible, enabling the bee to push the lip a little bit apart from the column, widening the exit so he can exit the bath. As he exits, he first passes the stigma. If he bears a pollinarium from a former visit, the bee presses the pollinia into the transverse stigmatic slit by its forward movement. The rostellum serves as a fork, catching the stipes and freeing the pollinia from the stipe and viscidium. As the bee struggles farther out the exit channel, the pollinarium is glued to the rear of his thorax. The pollinator struggles to get out of the flower, made more difficult by the slippery surfaces and lack of any structures that he can grasp with feet or mandibles (Plate 4). After successfully exiting the passage formed

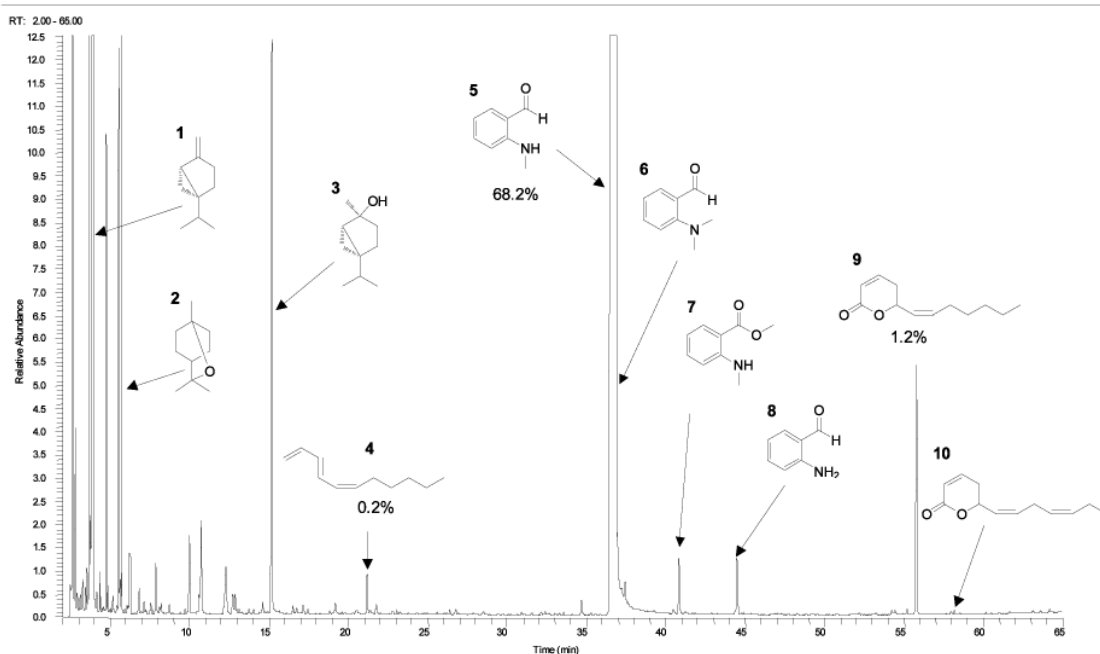


FIGURE 3. GC/MS analysis of the trapped scent of *Coryanthes kaiseriana*: 1 – Sabinene; 2 – Eucalyptol; 3 - trans-Sabinene hydrate; 4 - (E,Z)-1,3,5-Undeca-1,3,5-triene; 5 - 2-(Methylamino)benzaldehyde; 6 - 2-(Dimethylamino) benzaldehyde; 7 - Methyl N-methylanthranilate; 8 -2-Aminobenzaldehyde; 9 - (Z)-Dodeca-2,6-dieno-5-lactone; 10 - (Z,Z)-Dodeca-2,6,9-trieno-5-lactone. Courtesy of R. Kaiser (2006).

by lip and column, the bee remains on the flower for some time to dry itself. The entire pollination process lasts from 10 to 30 minutes depending on the presence or absence of the pollinarium.

Self-pollination is prevented by two mechanisms. First, the bee is probably traumatized by the bath and pollinarium placement and is less likely to revisit that same flower. Second, the fresh pollinia are too large to fit in the stigmatic slit and must dry for several minutes before they have shrunk enough to fit into the stigma.

The high degree of pollinator specificity mediated by floral fragrance composition may have several roles. First, it provides a reproductive isolating mechanism among sympatric species. Second, it reduces the number of bees of the wrong size from visiting the flower. This size specificity benefits both the bee and the orchid; if a bee too large for the exit canal is attracted and falls in the epichile, it cannot escape and dies in the flower, also preventing pollination. A bee too small for the flower can exit but does not effect pollination because it does not touch the stigma and pollinarium.

The floral fragrance of *Coryanthes kaiseriana* G.Gerlach (among other species) collected by the headspace technique includes two interesting chemical compounds (Fig. 3). 2-(methylamino)benzaldehyde (5) is a rare natural product, characterized by a peculiar dusty, leathery but still sweetish odor that is typical for *C. mastersiana* Lehm. (Gerlach & Schill, 1989; Kaiser, 2006). This species grows in the lowland forests and mangrove swamps of the Chocó region to the Cauca valley in Colombia and in northern Ecuador. We have analyzed numerous individuals of this species. The color variation is enormous (Plate 5), but the fragrance composition shows a significant homogeneity. In all clones investigated the fragrance is highly dominated by 2-(methylamino)benzaldehyde showing concentrations from 80-99%. Field studies carried out in Colombian Chocó near Bahia Solano with the synthetic product demonstrated the attractiveness of this substance to *Euglossa chalybeata* and *Euglossa asarophora*. Ten years after the discovery of this new natural product and five years after its description (Gerlach & Schill, 1989), we finally collected bees by using this compound as

PLATE 5. Variability within *Coryanthes mastersiana*, all plants from Colombia, Dept. Chocó. Pictures not to scale. (Photos: G. Gerlach)



Coryanthes mastersiana
Colombia, Dept. Chocó,
Pacific Coast



2-(Methylamino)-
benzaldehyde



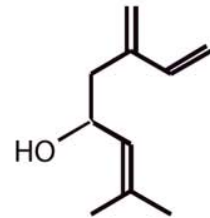
bait. Earlier attempts failed because we did not realize that this compound polymerizes easily, with resultant loss of activity. The substances 2-(dimethylamino) benzaldehyde (6), methyl N-methylantranilate (7), and 2-aminobenzaldehyde (8) are produced in the same biosynthetic pathway but have not been tested as attractants.

(E,Z)-1,3,5-Undeca-1,3,5-triene (4) is a highly fragrant representative of the other interesting group of chemicals in the fragrance of this *Coryanthes* species. This substance was found as a characteristic substance in the fragrance of *C. albertinae* Karsten from the Coastal Cordillera in Venezuela. Field tests in the habitat of that species with this synthetic alkene

PLATE 6. Different Stanhopeinae with ipsdienol as main substance; left above - *Sievekingia butcheri*; right above - *Trevoria glumacea*; middle - *Gongora lagunae*; left below - *Stanhopea anfracta*; right below - *Coryanthes trifoliata*. Pictures not to scale. (Photos: G. Gerlach)



Different Stanhopeinae
with Ipsdienol
as main component



failed; the whole forest was filled with the aroma of green apple, characteristic of that chemical, but not a single euglossine bee was attracted. Two other baits on the same morning attracted 12 bees carrying pollinaria of *C. albertinae*. The failure was explained 10 years later when an oxygenated compound with a much longer retention time was found by Kaiser (2006) in the fragrance of *C. albertinae*. His team identified it as (Z)-Dodeca- 2,6-dieno-5-lactone (9). Field tests with this substance revealed an attractant that was the most powerful we ever tested. One drop offered to the bees in the field lasted for nearly a week even when the bait was exposed to tropical rain or full sunlight. This persistence is due to the low volatility of the lactone. By contrast, eucalyptol (2), used by most researchers baiting euglossine bees, volatilizes quickly and lasts only about an hour, depending on the air temperature.

Within Stanhopeinae, similar floral fragrance patterns may occur in different genera. Because of differing floral morphology, the different genera attach their pollinaria at different sites on the bee's body. These plants may attract the same euglossine bee species as pollinators without the risk of producing intergeneric hybrids (which are possible if produced artificially), even when they are growing in the same habitat. In the examples below, mechanical isolating mechanisms (pollinarium shape and placement) are critical, but floral fragrances are nearly identical among distantly related taxa. Ipsdienol with its precursor myrcene (see also Whitten *et al.*, 1988) dominates the fragrance of *Stanhopea anfracta* Rolfe (40%/25%; ipsdienol/myrcene ratio of total composition), *Sievekingia butcheri* Dressler (75%/10%), *Gongora lagunae* G.Gerlach (40%/33%), *Trevoria glumacea* Garay (49%/16%), and *Coryanthes trifoliata* C.Schweinf. (51%/32%; Plate 6). These species place pollinaria at different sites on the bees: legs (*Sievekingia*); legs of presumably the right-hand side (*Trevoria*, never observed!); below the scutellum directed toward the abdomen (*Gongora*, *Stanhopea*); and below the scutellum but directed toward the head (*Coryanthes*). Similarly, methyl salicylate (wintergreen oil) occurs as large percentages in fragrances of *Houlletia lowiana* (84-98%), *Stanhopea candida* (55%), and *Coryanthes leucocorys* (76-95%), but each of these has distinctive pollinia attached in different locations or orientations on the bees.

I have touched only on the highlights of the interesting aspects of the ecology of *Coryanthes*. The different species are highly adapted to their habitats and consequently sensitive to human impacts on their environment. Protection of their natural habitats is the only way to conserve these strange plants. Hopefully, habitat conservation will ensure that our children will also be able to enjoy the study of *Coryanthes* in the wild.

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WHAT WILL BE LEFT OF THE PRIMARY FORESTS IN ECUADOR?

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ABSTRACT. Ecuador is among the smallest countries of South America (250,000 square kilometers) but has the highest density of human population with the highest network of primary and secondary roads, which also explains why Ecuador has the highest slash-and-burn ratio per capita in America (about 250,000 hectares per year). Over 20% of the country's surface has been declared as natural private and government reserves, where at least 2/3 of the area is totally inaccessible and has never been botanized. Thus, a large percentage of the approximate 1000 endemic orchid species described to date for Ecuador are probably not growing in these reserves. The remaining 80% of land is subdivided into 843,000 land ownerships, of which only 4% are parcels over 50 hectares. It can be concluded that no primary forests outside the reserves will survive the near future and respective endemic species will become extinct in nature. *Ex-situ* conservation is imperative.

RESUMEN. Ecuador es uno de los países más pequeños de América del Sur (250,000 kilómetros cuadrados) pero que tiene la más alta densidad de población humana con la red más alta de caminos primarios y secundarios, lo cual explica porqué Ecuador tiene la relación más alta per capita de corte y quema en América (aproximadamente 250,000 hectáreas por año). En exceso de un 20% de la superficie del país ha sido declarada como reservas naturales privadas o del gobierno, donde por lo menos 2/3 del área es totalmente inaccesible y nunca ha sido estudiadas botánicamente. Así, un gran porcentaje de las aproximadamente 1000 especies endémicas de orquídeas descritas hasta la fecha para el Ecuador, probablemente no estén creciendo en estas reservas. El 80% restante de la tierra se encuentra subdividida en 843,000 propiedades, de las cuales el 4% son parcelas de más de 50 hectáreas. Se puede concluir que ningún bosque primario que se encuentre fuera de las reservas podrá sobrevivir en el futuro cercano y que las respectivas especies endémicas se extinguirán en la naturaleza. Por lo tanto la conservación *ex-situ* resulta imperativa.

KEY WORDS: Conservation, forest, Ecuador

On December 14, 2008, the *New York Times Magazine* published an article (Risen, 2008) related to the rights of plants under the new Constitution of Ecuador. The article stated that Ecuador vaulted to the forefront of international ecopolitics when it became the first country to extend constitutional rights to nature, which includes the right to the maintenance and regeneration of its vital cycles, structures, functions, and evolutionary processes. The implications go much further, arguably granting broad protections to simple life forms such as algae and even bacteria.

In order to comprehend the challenges we face in the future, some important and relevant topics within the new constitution should be understood. The new constitution covers the rights of nature and its restoration in several chapters and at least 34 extensive articles. Also covered are Ecuadorians' rights for a

healthy, ecologically sustainable environment free of contamination and in harmony with nature. The State guarantees that, on a fair basis, citizens and communities will permanently have access to good quality water, air, soil, and to the benefits of the underground resources – otherwise known as the “natural patrimony.” Environmental preservation — including ecosystem conservation, all components of biodiversity, the integrity of genetic patrimony, the prevention of environmental damages, and the restoration of degraded natural areas — is declared to be of public interest.

Chapter VII is devoted entirely to the rights that nature has under this new Constitution, where Article 71 states the following: where life is generated and reproduced in nature, its existence has to be respected in its entirety, securing and maintaining its vital cycles,

structures, functions, and evolutionary processes. Any person or community may make demands to government authorities for the immediate fulfillment of these rights given to nature. Article 72 states that whatever party is responsible for damages to the environment must also be financially accountable for its restoration. They are also responsible to pay indemnities to whatever communities were affected by the damage. Articles 73 and 396 obligate the State to take the precautionary measures or restrictions to all activities that might lead to the extinction of species, the destruction of ecosystems or the permanent alteration of natural cycles. In case of doubt, even without scientific evidence for potential damage, the State has to adopt effective protective measures. Article 74 gives individuals and communities the right to benefit from the environment and its natural wealth to allow for better living. This is an interesting article in the Constitution because it implies that people and communities may, for example, collect orchids or insects in the wild for commercialization. This application of the article is a total contradiction to the current laws and regulations established by the Ministry of Environment.

Further on, a whole chapter is devoted to biodiversity and natural resources. In various Articles, it states that all environmental policies are obligatory for the citizens, organizations, and the State at all administrative levels. In case of doubt, the executive and judicial systems have to vote in favor of nature. Every person in the daily process of production, distribution, and use of goods and services assumes the responsibility to prevent any environmental impact, mitigate and restore any damages caused by his actions, and maintain a permanent environmental management program. Legal actions to prosecute and sanction the culprit for environmental damages cannot be waived under any circumstances.

Article 397 establishes the following: in case of environmental damage, the State will act immediately and subsidize the guarantees required for the restoration of health to the damaged ecosystem. The State will sanction not only the party responsible for damaging the ecosystem, but it will also make the public servants in charge of the environmental controls responsible for not preventing the damages in the first place. To enforce the above, any citizen or community

may exercise legal actions against parties who might endanger the health of any ecosystem, which includes water, air, soil, and the life forms within them. The accused party is responsible for proving its innocence. The government will also regulate and limit the landholdings in fragile ecosystems, which include the páramos, wetlands, cloud forests, tropical dry and wet forests, and mangroves. In relation to landholdings, Article 282 outlaws large landholdings. While Bolivia is considering reducing landholdings to between 5,000 and 10,000 hectares, in Ecuador the new legislation is considering limiting the landholdings to 800 hectares. It will also confiscate land that is not producing, which will raise questions from landowners who want to preserve natural habitats. In Article 411, the State guarantees the conservation, recuperation, and integral management of all water resources, water basins, and everything related to water cycles. In Article 414, the State will adopt adequate measures to mitigate the effects of global climate change by limiting gas emissions, deforestation, and air contamination. It will take measures for the conservation of the forests and other vegetation and protect the civilian population.

The title of this lecture is “What Will Be Left of the Primary Forests in Ecuador?” Under the new Constitution, all primary forests are protected, and any damage has to be restored immediately by all levels of government. Article 426 states that applicability and observance of this Constitution is in force immediately, where the authorities may not delay any action described in the new Constitution by claiming that the bylaws and regulations are not yet in place or invoking any other excuse. It is generally agreed that about 300,000 hectares of primary ecosystems are burned down every year in Ecuador. Any person, organization or community may now demand that the State immediately restore any destruction and punish the culprits.

In his article published by the *New York Times Magazine*, Risen (2008) pointed out that it's uncertain how, exactly, a country as poor as Ecuador can protect these rights and how it can protect *Pachamama* or Mother Universe and the ecosystem as a whole. With a surface of only 283,561 km², Ecuador is second smallest among the countries of South America, after Uruguay. Ecuador has the highest density of human population in South America with 49 inhabitants per

km², whereas Peru, a country five times larger than Ecuador, has 23 inhabitants per km², and Bolivia, a country four times larger, has only 8.3 inhabitants per km². Ecuador has 14 million inhabitants living in the country and another three million working abroad. About 37% live in rural areas. The birthrate is 1.5%, which means that every year 210,000 Ecuadorians turn 18 and enter the work force. It is difficult for any government to secure 210,000 additional jobs per year, and Ecuador has to assume that, of the three million working abroad, many will return to Ecuador over the next two years because they lost their jobs in the global economic crisis. Ecuador has the highest network of primary and secondary roads in South America, adding up to at least 40,000 kilometers. Ecuador is only 700 kilometers long, north to south, from the border of Colombia to the border of Peru. The growing capital city of Quito already covers 7% of this distance.

These area constraints and population density factors are the main reasons why Ecuador has the highest ratio of slash-and-burn to total surface area of any country in the Americas; we lose around 300,000 hectares of primary forests per year. Because of the high density of plants growing on trees, about 20 to 30 million plants are burned every hour. The likelihood of our generation seeing a dramatic decrease in the rate of deforestation is near impossible. Decreeing a new constitution where nature has almost unlimited rights is a futile effort, because it will only slow down economic growth and force the poor to move into the remaining forests. The rural population would have to be educated in record time and given realistic alternatives for their survival in the next five years, because in 10 years there will be no forests left to save. In the efforts to save nature, most conservationists fight only mining and oil companies and rarely address the real problem, which is extensive subsistence agriculture practiced by the poor. The efforts of a handful of concerned botanists trying to stop the ancestral practice of slash-and-burn agriculture will not change the fact that it is an enormously successful adaptation to the rigors and constraints of the tropical forest.

Ecuador is fortunate to have over 20% of its surface declared as natural private and government reserves, within which at least two thirds of the areas are totally inaccessible and have not even been botanized. Unfortunately, a paragraph in Article 405 of the new

Constitution states that neither a foreigner nor any foreign organization may own title to any property or concessions in protected areas. This will undoubtedly raise legal issues in cases where foreigners or NGOs have previously purchased land and had it declared as a protected area, only to lose it under the new Constitution.

A large percentage of the approximate 1,000 endemic orchid species described to date for Ecuador are probably not growing in the currently established natural reserves. Almost all of these described species were made from collections on private property next to the road. The remaining 80% of land in Ecuador is subdivided into 843,000 land ownerships, where only 4% are parcels over 50 hectares. This 4% of landholdings will soon be subdivided under the new Constitution, probably into areas no larger than 500-800 hectares. Officials of the current government have also stated they will not tolerate unused land and give it to people in need. That implies that whoever has any primary forest left had better replace it with pasture, as was customary in the 1960s and 1970s under the Agrarian Reform. Of course, this threat directly contradicts the new Constitution.

It can be concluded that no primary forests outside the reserves will survive in the near future, and respective endemic species in these forests will become extinct in nature. To make matters worse, even if we optimistically assume that farmers and loggers will not impact the protected areas in the coming decades, we cannot blindly believe that the orchid species living in these forests will survive the oncoming climate changes, regional acid rains, and dehydration from the surrounding desertification. To secure the biodiversity of the protected areas, we cannot ask for more than what is already written in the new Constitution. But it is clear that the other forests or biodiversity on private property will not be around for much longer. Therefore, *ex-situ* conservation measures have to be taken more seriously, particularly with the implementation of botanical gardens distributed at all levels throughout the country.

Tourism directed to bird-watching, butterfly farms or orchid photo safaris is one of the main methods to sustain biodiversity; local communities will quickly learn that this will bring business and well-being to their people. Many small communities have already

started to plant native species in their gardens or public places, particularly orchids and bromeliads that they rescued from fallen trees. The Ministry of Environment is assisting such communities in their efforts to turn this into a legal activity, where the growers of these botanic gardens obtain the respective permits and authorizations. The communities of the “Nor-Occidente” around Mindo and San Miguel de los Bancos are good examples.

This year the Orchid Society of Quito has decided to patronize the implementation of the orchid garden “Sisa Ricsina, Flowering in Community.” This project was proposed and will be managed by a network of six native Kichwa communities in the province of Napo that are currently forming the Mushuk Sisa Foundation. These communities are already well organized and successful in community-operated tourism. Their botanical garden, highlighting orchids, will be established next to the Río Jatun Yacu.

We of the Quito Orchid Society are dedicated to strengthening initiatives that will protect our environment and educate our people. We take it as our duty to navigate through the restrictions and freedoms in Ecuador’s new Constitution. With pride and diligence we will accept the challenge and succeed in helping orchids survive and be enjoyed by everyone. It is our hope that other orchid societies both here in Ecuador and abroad will be able to face our collective challenge and help sponsor small communities in their nascent botanical garden projects. These communities are our keys to success and the future of orchid tourism and orchid *ex-situ* conservation.

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HOW UNIFORM IS SPECIES DIVERSITY IN TROPICAL FORESTS?

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ABSTRACT. Questions concerning species diversity have attracted ecologists for over a century. One of these factors is the sampling effort - the “botanist effect,” which can be, for example, the number of orchidologists in the region. The sampling effort can also account for the fact that most endemic orchid species are found close to roads, which indicates that orchid diversity may decline from the forest edge towards its interior. Here we tested this hypothesis, using data on orchid species from Bolivia. We found opposite trends in terrestrial and epiphytic species. Both species diversity and number of individuals of terrestrial species declined toward the forest interior. However, for epiphytic species, both species diversity and number of individuals of species increased toward the forest interior. Only because of the prevalence of the terrestrial species did the total number of species and the total number of individuals decline towards the forest interior. Thus, when making conclusions about the trends in orchid diversity towards the interior of the forest, their life mode should be taken into account. The reasons for the trends observed are quite straightforward. Toward the forest interior, density of the trees increases, and therefore the amount of light available on the ground declines. Hence, habitats close to the openings (roads, meadows, fields, etc.) are more suitable for terrestrial species, whereas those deep in the forest interior are more suitable for epiphytic species because of the availability of host trees.

RESUMEN. Las preguntas acerca de la diversidad de especies ha atraído a los ecologistas desde hace más de un siglo. Uno de los factores que genera preguntas es la distribución de las especies, misma que se podría ser un resultado del llamado “efecto de los botánicos” o efecto del esfuerzo de colección, así por ejemplo una zona donde existan muchos orquideólogos, con seguridad tiene una gran diversidad, pero al mismo tiempo gran esfuerzo de colección. Otro fenómeno común asociado al esfuerzo de colección de encuentra en la distribución de especies endémicas, que aparecen como más diversas al borde de los carreteros, y que declinan su diversidad hacia el interior del bosque. En este estudio probamos esta hipótesis utilizando datos de especies de orquídeas bolivianas. Se encontró diferencias entre especies terrestres y epífitas. En el caso de las terrestres, tanto la diversidad como el número de individuos disminuyó hacia el interior del bosque. Sin embargo, las especies epífitas incrementaron su abundancia y diversidad hacia el interior del bosque. Las razones que explican los patrones observados son evidentes, ya que hacia el interior del bosque hay mayor densidad de árboles por lo tanto hay menor cantidad de luz que llega al suelo, por lo cual la diversidad de las especies terrestres declina. Así, los hábitats cercanos a claros de bosque son más propicios para especies terrestres, mientras que los que se encuentran al interior del bosque son apropiados para las especies epífitas debido a una mayor disponibilidad de árboles hospederos.

KEY WORDS: Orchidaceae, species diversity, sampling effort, tropical forest, Bolivia

Questions concerning species diversity have attracted ecologists for over a century (Sch. delbauerov. *et al.*, 2009). Increase in species richness from the poles to the tropics (Pianka, 1966; Rohde, 1992; Willig *et al.*, 2003; Hillebrand, 2004) and with area (Arrhenius, 1921; Gleason, 1922; Williamson, 1988; Rosenzweig,

1995) is still one of the main topics in contemporary ecology. More recently, the amount of energy available (i.e., that which can be converted into biomass) for net primary productivity has been revealed to be an important determinant of species richness (Wright, 1983; Wylie & Curie, 1993a,b; Pelkey *et al.*, 2000;

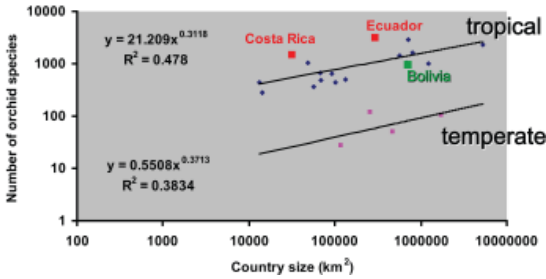


FIGURE 1. Numbers of orchid species in various countries of the whole of Latin America plotted against size of each country. Different dependences hold for tropical and temperate countries: the fitted lines are almost equally steep, but that for the tropical countries lies far above that for temperate ones. The R^2 values are coefficients of determination, denoting the proportion of variability in the data explained by the fitted line (53% and 25%, respectively, in our case).

Evans *et al.*, 2005; Storch *et al.*, 2005). Area is clearly the most influential determinant, but other factors may also be important.

One of these factors is the sampling effort - the “botanist effect” — which is related to, for example, the number of orchidologists in the region. The “botanist effect” is thought to be the reason for higher plant species richness in areas where botanists are disproportionately present as an artefactual consequence of a more thorough sampling (Pautasso & McKinney, 2007). For orchids this is illustrated in Figure 1 using the examples of Ecuador, Costa Rica, and Bolivia, which are similar to each other in many respects: they are tropical, mountainous countries with similar diversity of habitats and similar climatic conditions. Ecuador and Costa Rica were well studied and are above the regression line. There is much less known about orchid species in Bolivia, which is below the line.

The sampling effort can also cause the effect observed by Endara *et al.* (2007) – most endemic orchid species were found close to the roads. Thus it seems that orchid diversity declines from the forest edge towards its interior. Here we test this hypothesis, using data on orchid species from Bolivia.

Description of study area

Our study area covered approximately 80 hectares of the Uchumachi mountain close to the settlement of Carmen Pampa (16°20’30” S, 67°50’00” W) in the



FIGURE 2. The approximate position of the study area is indicated here by the red dot.



FIGURE 3. The the study area.

municipality of Coroico, province of Nor Yungas, department of La Paz, Bolivia, in the eastern slopes of the Andes.

The elevation of our plots ranged from 1957 to 1995 meters above sea level. The eco-region here, called *yungas*, is characterized by mountain chains with wide slopes and long valleys formed from sedimentary and metamorphic rock (Fig. 2). Elevations range from 400 to 2800 meters above sea level (Morales, 2004). The study area consisted of the road verge, secondary forest, and primary forest. The secondary forest hosts a great diversity of species, including tree ferns (*Cyathea amazónica* Domin), “sikilis” (*Inga* sp.), walnut (*Juglans boliviana* Dode), “ambaibos” (*Cecropia angustifolia* Tr.cul), and a diversity of ferns, mosses, and palms. It is characterized by a

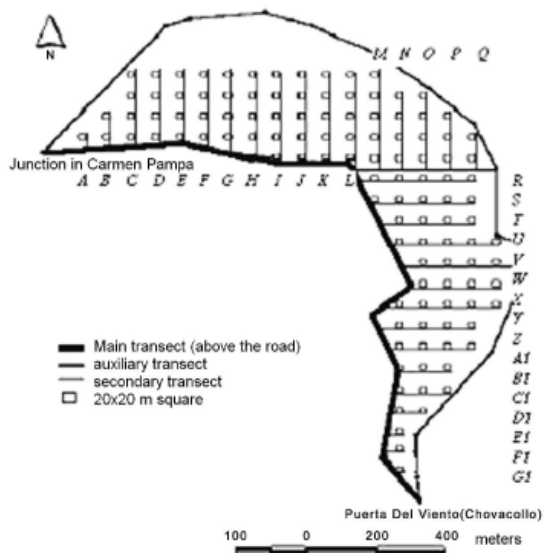


FIGURE 4. Plan of the study area. The thick black line is the road. Thin lines indicate the transects, and small circles are the plots.

dense understory with evidence of selective logging. The primary forest is characterized by the presence of taller trees, wider in diameter, and a reduced understory due to a reduction of light in the forest floor. Human activity is also much reduced here. This forest is dominated by tree ferns (*Cyathea amazónica*), individuals from Lauraceae, “espeke” (*Clusia haughtii* Cuatrec), “leche leche” (*Sapium aereum* Klotzsch ex Müll.Arg.), and “mata palo” (*Ficus obtusifolia* Roxb.) that can reach diameters of over 100 cm and account for a large part of the basal area. Other species such as “jaluti” (*Miconia guianensis* Cogn.), wild papaya (*Oreopanax* sp.), and “suti suti” (*Miconia minutiflora* DC.) are found at densities of one or less per hectare, which indicates that they may be under the threat of extinction (Endara, 2001).

The average of ten years of meteorological data from an on-site weather station shows that the average temperatures in the warmest month (January) are above 19 C and in the coldest months (June and July) about 15 C, with maximum yearly temperatures around 25 C and minimum yearly temperatures of 15 C. The total annual precipitation is 2390 mm, with maximum precipitation from December to April (200-300 mm per month) and no month with less than 80 mm. Relative humidity is between 50-100%.

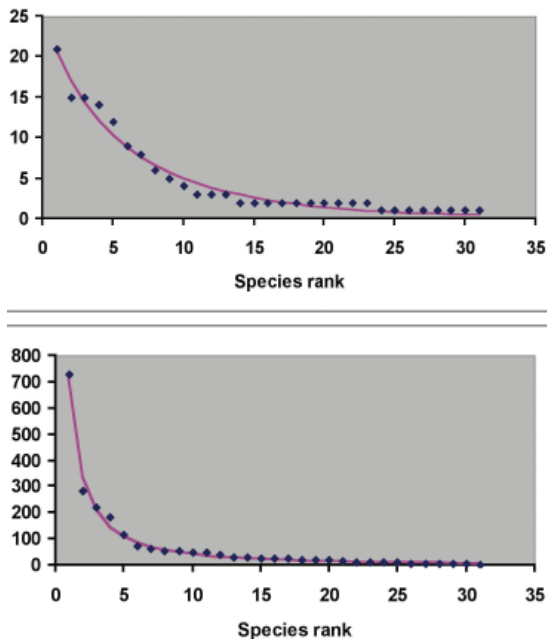


FIGURE 5. Number of squares in which the species was found (top) and number of individuals of the species (bottom) against the species rank, when the species are ranked from the commonest to the rarest.

Methods

We used linear transects perpendicular to the road depicted in Figure 3. Along the transects, we set up 117 evenly spaced 20x20 m plots, in which all orchid species were determined. The accuracy of species identification was verified using the orchid collection in the Herbario Nacional de Bolivia. Out of the 117 plots, 33 were adjacent to the road (habitat R), 40 were in the secondary forest (habitat S), and 44 were in the primary forest (habitat P; see Fig. 4).

Results

Altogether, 2159 individuals were found in the 117 plots. The orchids showed the classical negative exponential pattern, when the species were ranked from the commonest to the rarest (Fig. 5). Table 1 shows the parameter values (a, b, c) and residual sums of squares (RSS) of the function $y = a \cdot e^{-bxc}$, fitted to the relationship between the number of squares in which the species was found and species rank, when the species are ranked from the commonest to the rarest (“Squares”) and those of the function $y = a \cdot e^{-bxc}$, fitted to the relationship between the

TABLE 1. Parameter values (a, b, c) and residual sums of squares (RSS) of the function $y = a.e^{-bxc}$, fitted to the relationship between the number of squares in which the species was found and species rank, when the species are ranked from the commonest to the rarest (“Squares”) and those of the function $y = a.e^{-bxc}$, fitted to the relationship between the number of individuals of the species and species rank, when the species are ranked from the commonest to the rarest (“Individuals”).

	Individuals	Squares
a	286625	26.55
b	6.00	0.25
c	0.17	0.83
RSS	5288.1	21.8

number of individuals of the species and species rank, when the species are ranked from the commonest to the rarest (“Individuals”). Table 2 shows the species found in individual habitats. Out of the total of 31 species (13 epiphytic and 18 terrestrial species), only one species occurred in all three habitats, the epiphytic *Pleurothallis xanthochlora* Rchb.f. There were 3 epiphytic and 6 terrestrial species, which occurred in two habitats. The remaining 9 epiphytic and 12 terrestrial species were

specialized to only one of the habitats.

Figure 6 shows the average number of species per square and average number of individuals per square that were found at the edge of the road, in the secondary forest, and in the primary forest for terrestrial and epiphytic species. Clearly, both the number of terrestrial species and the number of individuals of terrestrial species strongly decreased toward the interior of the forest. The largest number of individuals and species was in the road verge, followed by the secondary forest, and finally the primary forest with the smallest number of terrestrial species and individuals. An opposite situation occurred in epiphytic species. Both the number of epiphytic species and the number of individuals of epiphytic species strongly increased toward the interior of the forest. The smallest number of individuals and species was in the road verge, followed by the secondary forest, and the primary forest with the largest number of epiphytic species and individuals.

Discussion and conclusions

We found opposite trends in terrestrial and epiphytic species. Both species diversity and number of individuals of terrestrial species declined toward

TABLE 2. Alphabetical list of epiphytic and terrestrial species found in individual habitats (R – road verge, S – secondary forest, P – primary forest). Species common to all three habitats is in red and highlighted in yellow; species common to two habitats are highlighted in blue.

Epiphytes	R	S	P	Terrestrials	R	S	P
<i>Acianthera heliconioides</i> (Luer & R.Vásquez)				<i>Bletia catenulate</i> Ruiz & Pav.	38	0	0
Pridgeon & M.W.Chase	0	0	4	<i>Elleanthus hookerianus</i> (Barb.Rodr.) Garay	3	0	0
<i>Cyrtorchilum myanthum</i> (Lindl.) Kraenzl.	0	0	8	<i>Epidendrum funckianum</i> A.Rich. & Galeotti	691	37	0
<i>Epidendrum jajense</i> Rchb.f.	8	0	5	<i>Epidendrum secundum</i> Jacq.	27	0	0
<i>Epidendrum macrocarpum</i> Rich.	0	2	7	<i>Stelis</i> sp. 2	26	47	0
<i>Epidendrum tridens</i> Poepp. & Endl.	2	0	0	<i>Habenaria macronectar</i> (Vell.) Hoehne	26	0	0
<i>Notylia peruviana</i> (Schltr.) C.Schweinf.	9	0	0	<i>Koellensteinia boliviensis</i> (Rolfe ex Rusby) Schltr.	9	0	0
<i>Oncidium tigratum</i> Rchb.f. & Warsz.	0	0	17	<i>Maxillaria aggregate</i> (Kunth) Lindl.	6	0	0
<i>Pleurothallis cordata</i> (Ruiz & Pav.) Lindl.	0	0	5	<i>Maxillaria longicaulis</i> Schltr.	3	0	0
<i>Pleurothallis linguifera</i> Lindl.	0	21	0	<i>Ornithidium aureum</i> Poepp. & Endl.	52	0	0
<i>Pleurothallis xanthochlora</i> Rchb.f.	46	76	60	<i>Polystachya boliviensis</i> Schltr.	28	0	0
<i>Restrepia antennifera</i> Kunth	0	0	114	<i>Sobralia bletiae</i> Rchb.f.	16	3	0
<i>Scelochilus laeae</i> Dodson & R.Vásquez	0	0	24	<i>Sobralia dichotoma</i> Ruiz & Pav.	46	0	0
<i>Stelis</i> sp. 1	0	33	16	<i>Sobralia dorbigniana</i> Rchb.f.	24	0	0
				<i>Sobralia fimbriata</i> Poepp. & Endl.	148	73	0
				<i>Sobralia</i> sp.	0	37	17
				<i>Sobralia yauaperyensis</i> Barb.Rodr.	258	0	26
				<i>Zygopetalum maculatum</i> (Kunth) Garay	61	0	0

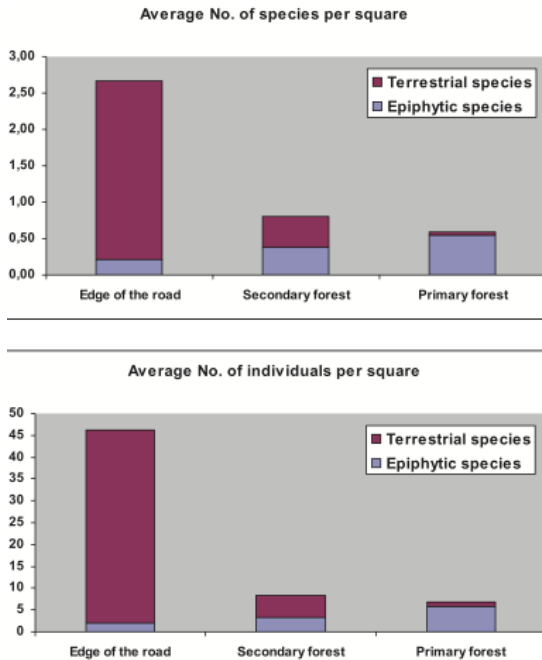


FIGURE 6. Average number of species per square (top) and average number of individuals per square (bottom) that were found at the edge of the road, in the secondary forest, and in the primary forest for terrestrial and epiphytic species.

the forest interior. However, in epiphytic species both species diversity and number of individuals of epiphytic species increased toward the forest interior. Only because of the prevalence of the terrestrial species did the total number of species and the total number of individuals decline toward the forest interior. Thus, when making conclusions about the trends in orchid diversity toward the interior of the forest, their life mode should be taken into account. The reasons for the trends observed are straightforward. Toward the forest interior, density of the trees increases, and therefore the amount of light available on the ground declines. Therefore, habitats close to the openings (roads, meadows, fields, etc.) are more suitable for terrestrial species, whereas those deep in forest interior are more suitable for epiphytic species due to the availability of host trees.

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ACTIVE MOUNTAIN BUILDING AND THE DISTRIBUTION OF “CORE” MAXILLARIINAE SPECIES IN TROPICAL MEXICO AND CENTRAL AMERICA

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ABSTRACT. The observation that southeastern Central America is a hotspot for orchid diversity has long been known and confirmed by recent systematic studies and checklists. An analysis of the geographic and elevation distribution demonstrates that the most widespread species of “core” Maxillariinae are all adapted to life near sea level, whereas the most narrowly endemic species are largely distributed in wet highland environments. Drier, hotter lowland gaps exist between these cordilleras and evidently restrict the dispersal of the species adapted to wetter, cooler conditions. Among the recent generic realignments of “core” Maxillariinae based on molecular phylogenetics, the *Camariidium* clade is easily the most prominent genus in Central America and is largely restricted to the highlands of Costa Rica and Panama, indicating that this region is the ancestral home of this genus and that its dispersal limits are drier, lowland cordilleran gaps. The mountains of Costa Rica and Panama are among the geologically youngest topographic features in the Neotropics, reflecting the complex and dynamic interactions of numerous tectonic plates. From consideration of the available geological evidence, I conclude that the rapid growth of the mountain ranges in Costa Rica and Panama during the late Cenozoic times created, in turn, very rapid ranges in ecological life zones and geographic isolation in that part of the isthmus. Thus, I suggest that these recent geologic events were the primary drivers for accelerated orchid evolution in southeastern Central America.

RESUMEN. Desde hace muchos años, observaciones indican que sur-este de América Central es un punto de alta diversidad de orquídeas. Éstas han sido confirmadas recientemente por estudios en la sistemática y listas anotadas este grupo. Un análisis de la distribución geográfica y altitudinal, demuestra que las especies más ampliamente distribuidas del “core” Maxillariinae se encuentran adaptadas para desarrollarse en tierras bajas cerca del nivel del mar, mientras que las especies endémicas y con distribuciones restringidas se encuentran en zonas altas y húmedas. Entre los recientes re-arreglos del “core” Maxillariinae, basado en filogenética molecular, aparece que el caldo *Camariidium* es el género más prominente en América Central, y está restringido a las tierras altas de Costa Rica y Panamá, indicando además que esta región es su lugar de origen y que sus límites para su dispersión son las tierras bajas y más secas. Las montañas de Costa Rica y Panamá se encuentran entre los accidentes geográficos más jóvenes del Neotrópico, y es el resultado de la compleja y dinámica interacción de numerosas placas tectónicas. Considerando la información geológica disponible, se concluye que el rápido crecimiento de las cadenas montañosas de Costa Rica y Panamá durante el Cenozoico Tardío, produjo rápidos cambios en las zonas ecológicas y también aislamiento geográfico en parte del istmo centroamericano. Así, se sugiere que estos eventos geológicos recientes fueron los factores primarios que aceleraron la evolución de las orquídeas en el sur-este de América Central.

KEY WORDS: Central America, geology, geography, Orchidaceae, Maxillariinae, distribution

Charles Darwin geologist

In this bicentenary year of Charles Darwin’s birth, it is noteworthy to commemorate his seminal research relating earthquakes and mountain building to

environmental change and the biogeography of South America. The purpose of this paper is to apply and extend some of Darwin’s concepts and observations to provide geological context and explanation for the reasons why southeastern Central is such a hotspot

for orchid evolution. However, before I discuss the interplay between mountain belts (and associated lowland gaps), geologic processes, and biological diversity, it is important to review briefly the life and contributions of Charles Darwin as the emerging science of geology developed in the 19th century.

It is sometimes forgotten that Charles Darwin, a founding father of modern biology, was first and foremost a naturalist and that early in his life as a scientist he made important contributions to geology. In late December of 1831, the 22-year-old Darwin (1809-1882), a freshly minted graduate of Cambridge University, sailed on the British Royal Navy ship, *HMS Beagle*, on an around-the-world voyage of exploration and discovery. Although an avid student of the biological world, he was expected to serve also as the shipboard naturalist, and in this capacity he was given by Captain Robert FitzRoy (1805-1865) the first of three volumes of the first edition of Charles Lyell's (1797-1875) famous series, *The Principles of Geology* (1830, 1832, 1833), a landmark treatise about the rock record as interpreted in terms of present-day processes: "The present is the key to the past" (Lyell, 1830). Darwin had also received brief field training in geology from Adam Sedgwick (1785-1873) in late summer of 1831. With this limited knowledge and experience in geology, but with a keen observer's eye and a developing willingness to create conceptual models of how nature works, he explored the globe with his shipmates. In the decade after the conclusion of the *Beagle's* voyage in 1836, he wrote several important books and many journal articles that became founding documents in the 19th century development of the geological sciences (e.g., Darwin, 1838, 1839, 1842, 1844, 1846). In three of these publications (Darwin, 1838, 1839, 1844), he described several independent observations that he made during the *Beagle's* exploration of the Pacific coastal Andes. First, he documented the numerous formerly marine terraces raised by as much as 400 meters above sea level. These terraces were marked with shallow-water marine seashells that were indistinguishable from those living offshore and with the lower terraces having less weathered shells than the higher ones, indicating relatively recent uplift over a period of recent geologic time. On February 20, 1835, Darwin and his field assistant debarked from the *Beagle* at anchor in Valdivia, Chile (Yeats *et al.*, 1997). At about 11:40

a.m. local time, a great subduction earthquake occurred offshore of south-central Chile north of Valdivia. Strong ground motion lasted two minutes; the near-shore area of the town was damaged due to the seismic sea waves, and the *Beagle* was hit with jarring motions as if it had run aground (Yeats *et al.*, 1997; Herbert, 2005). With Darwin and his assistant back on board, Captain FitzRoy weighed anchor and sailed north to the source area. Dropping anchor at Talcahuano, the port town serving the city of Concepcion, the party observed a large region of coastal uplift that was produced by the earthquake. Although these uplifts are sudden during earthquakes, such shocks occur infrequently and thus integrated over time; average uplift rates are of the order of just millimeters per year or less. Later, Darwin led a mule-pack-train exploration of the high Andes to the passes between present-day Santiago and Portillo in Chile and Mendoza, Argentina, traveling beneath the foot of Aconcagua, the highest peak in the Andes. During this journey he collected rocks with marine fossils at elevations of between 3,000 and 4,000 meters. Based on these three observations, he hypothesized that the earthquakes somehow represented the motions that raised the shorelines, produced the raised marine terraces, and slowly built the Andes mountain range (Darwin, 1839, 1844).

Darwin was not the first to make observations like these, but he apparently was among the first to put them all together. It would be decades later before it was generally understood that most tectonic cordilleras like the Andes are a consequence of horizontal compressive deformation or shortening, leading to thrust-faulting and folding that elevated continents during shortening and consequent thickening (e.g., Fisher, 1881; Suess, 1883-1909), and that continuous belts of earthquakes are often coincident with or parallel to such mountains (Mallet, 1858; Milne, 1886). It was more than a century later before it was understood that compressive deformation of continental margins is generally a consequence of subduction motion of oceanic plates as they collide with the upper plate and sink into Earth's mantle or of collisions between continents or elevated seafloor features with continents (Coats, 1962; Oliver and Isacks, 1967; Isacks *et al.*, 1968; Dewey and Bird, 1970). Darwin's sweeping chain of logic and insightful inference has proven to be an important principle of modern investigations of the tectonics of present-day

mountain building: **earthquakes mark the places near where tectonic mountains are being built.** In the context of modern digital seismology, earthquakes can be monitored anywhere in the world using global stations, complete down to about Richter magnitude 5. Satellite (GPS) measurements of earth movements also allow us to pinpoint over time where active mountain building is occurring at present. Likewise, volcanic eruptions along the spine of the Andes and volcanic cordilleras elsewhere in Latin America mark places where active mountain building by volcanic construction is taking place. These processes of active mountain building, although generally slow by human standards, can change the distribution of climatic conditions over geologic time, such as rainfall patterns and average diurnal temperatures, and other climatic factors that can potentially affect biological evolution. Moreover, Darwin noted the marked differences in biota on each side of the Andes, an observation that indicated to him that this mountain range represented a barrier to species migration. Thus Charles Darwin's early work as an insightful pioneer in geology also has a direct bearing on our understanding of one of the causes of environmental change that, in turn, is one of the principal drivers for speciation and biological evolution through natural selection that he also pioneered along with Alfred Wallace (Darwin, 1858; 1859, 1862; Wallace, 1858, 1870, 1889).

This paper is a preliminary exploration of these concepts as applied to southeast Mexico and Central America during the bicentenary year of Darwin's birth and the 150th anniversary of the publication of *Origin of Species*. It is intended as a preliminary application of knowledge of the tectonic and volcanic events of this region that is possibly relevant to the geologically recent changes in environmental conditions that may have shaped in part the distribution of orchid species and their evolution during the same period of geologic time of the evolution of our own genus, *Homo*, in Africa.

Southeast Central America: a hotspot of orchid diversity

The status of southeast Central America as a biological hotspot has long been recognized (see reviews by Dressler, 1985; Burger, 1985; Myers *et al.*, 2000; Ossenbach, 2009). Although there are strong differences in the likely degree of undersampling of

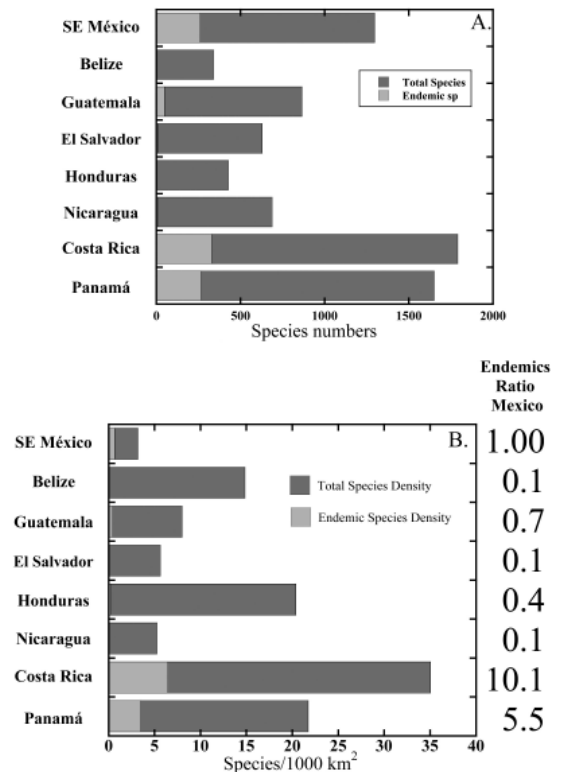


FIGURE 1. Distribution of orchid species numbers by geographic area (data source: Ossenbach *et al.* (2007)). A. Total species and endemic species numbers by country. B. Species per 1000 km² country area (species density) for both total species and endemic species.

orchid flora and the degree of deforestation and habitat loss by country, the data from a recent checklist of orchid species distribution in Central America and southeast Mexico show that differences in total orchid diversity and endemic species per country area (Fig. 1A, B) are extremely large (Ossenbach *et al.*, 2007), probably more than expected due to differences in the degree of undersampling. In particular, the species inventories of Costa Rica and Panama show manifold area density contrasts with the rest of tropical America in the Northern Hemisphere. In general, country borders are usually not natural biogeographic boundaries, but orchid species distributions are often known only to the country level, so we are forced in our analysis to restrict ourselves to this crude breakdown. Nonetheless, there are stark differences between the large area densities of endemic orchid species of Costa Rica and Panama with their cordilleras and intermontane valleys on the

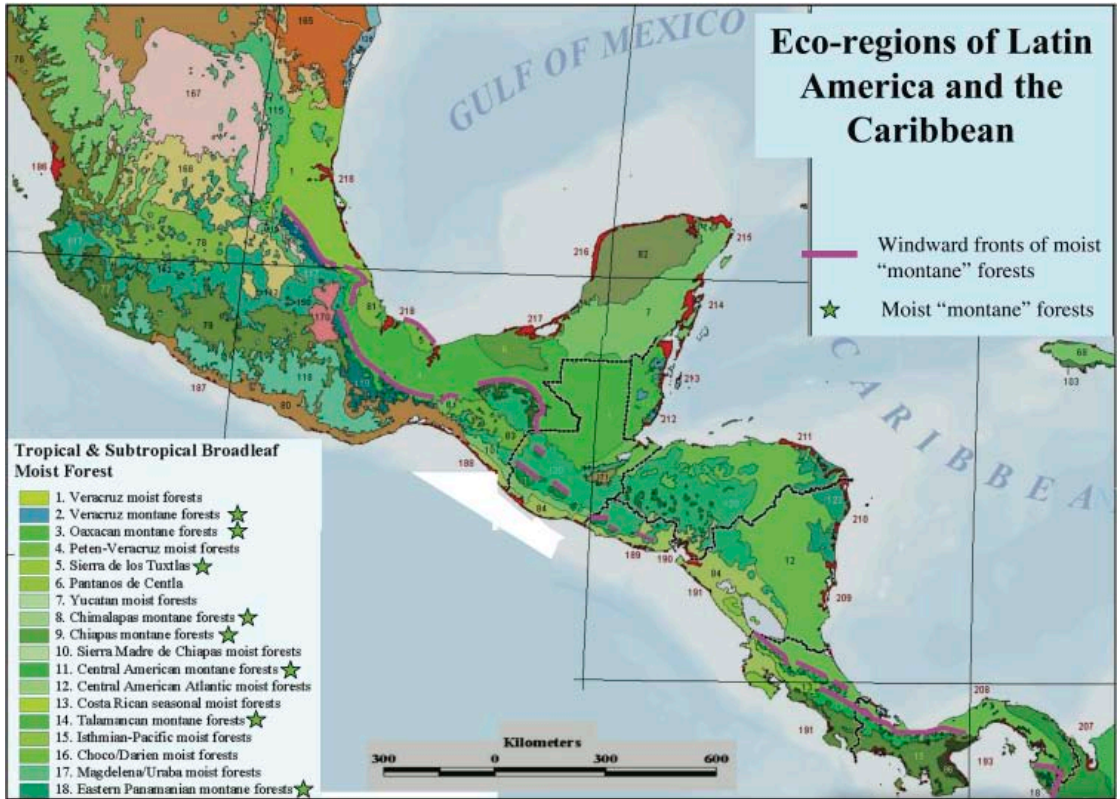


FIGURE 2. Map showing the eco-regions of Central America and southeast Mexico with emphasis on moist, broadleaf, tropical/subtropical forests. Magenta lines mark the approximate windward fronts of wet upland life zones. Modified from the online Nature Conservancy Landscape Ecology Program Map: "Ecoregions of Latin America and the Caribbean" [Dienerstein *et al.* (2001)].

one hand and the low species densities of the largely lowland countries of Nicaragua, El Salvador, and Belize, differences that are impressive and probably transcend sampling biases. An important question is why such differences exist, an enigma that is the subject of this paper, one that builds on the work of Dodson (2003) concerning origins of the diversity of orchids in Ecuador. His hypotheses are largely echoed and amplified in the present paper and applied to southeast Central America.

Mountain belts, geological processes, and biological diversity

It is well established that wet tropical premontane to montane life zones are among the most diverse and biologically exuberant regions in the world (e.g., Holdridge, 1947, 1967; Hall and Brignoli, 2003; Ossenbach, 2009). By implication, such zones are also

crucibles for biological evolution. Mountains create regional climatic conditions through the orographic effects of adiabatic cooling of moist air moving upslope that increase rainfall and also the effects of enhanced radiative cooling of thinner atmospheres at higher elevations in moderating tropical temperatures and promoting plant growth. The mountain belts in Central America form discontinuous curvilinear belts of moist premontane to montane forests from southern Mexico to Panama (Fig. 2).

I discuss below other effects of mountain chains (and processes that occur in them) on the biogeography of tropical orchids within the Americas in the context of the major governing biogeographic processes: **dispersal, speciation, and extinction** (Fig. 3). I consider these processes in this context of the ensemble of orchid species and their biological cohorts — their pollinators and the mycorrhizal fungi that

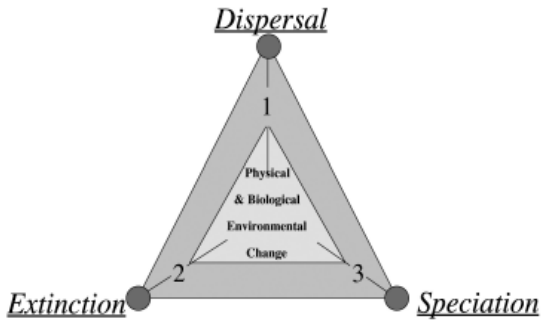


FIGURE 3. Schematic ternary diagram depicting graphically the links (1, 2, and 3) between the drivers of evolution of biota (physical and biological changes) and the processes that control the biogeography of life forms: dispersal, speciation, and extinction. See text for discussion.

enable them to germinate. It should be understood that any biogeographic conclusions reached in this report pertain to the ensemble of these cohorts.

The spectrum of active geological processes, environmental change, and evolution: dispersal, extinction, and speciation

Dispersal—That mountain belts serve as partial barriers or ‘filters’ to gene flow of orchid species adapted to lowland tropical conditions is obvious from inspection of the orchid flora of countries of the tropical Americas, especially in differences between the flora of the Caribbean and Pacific slopes in Central America and the Atlantic (Amazonian) and Pacific slopes in South America. Mountains in the tropics represent more effective filters to migration of species adapted to lowland conditions, because lowland tropical species are not forced to adapt to large seasonal temperature fluctuations as lowland plants must at high latitudes, i.e., in the tropics, montane and lowland temperatures do not overlap with changes in the seasons (Janzen, 1967). Conversely, hot, dry lowlands interrupting mountain chains can serve as partial barriers to dispersal of orchid species adapted to cool, moist highland conditions. I use this term ‘partial’ in light of rare, long-distance orchid dispersal events that have led to occurrence of orchid species on Cocos Island, some 530 km from mainland Costa Rica, some of which are endemic to the island and some found elsewhere in Central America and Peru (Trusty and Blanco, 2005; Trusty *et al.*, 2006).



FIGURE 4. Color-shaded relief topography of southeast Mexico, Central America, and northwest Colombia showing the principal lowland gaps between cordilleras.

The major lowlands that interrupt southeast Mexican and Central American cordilleras are from north to south (Fig. 4): 1) the Tehuantepec (Chiapas/Oaxaca) gap; 2) the Nicaraguan depression; 3) the Gatun-Balboa gap crossed by the Panama Canal; 4) the valley of the R.o Chepo that separates the Pacific and Caribbean coast ranges in eastern Panama; 5) the R.o Chucuaque/Gulf of San Miguel gap; and 6) the near-coastal Colombian lowland between the Gulf of Urab. and Buenaventura Bay in Colombia that separates the Pacific coast ranges of Panama and northwest Colombia from the main Andean cordillera (the Cordillera Oriental). The Nicaraguan depression is easily the most prominent lowland gap among mountain ranges in Central America based on its width and the fact that some of this lowland is occupied by the lowland Lakes Nicaragua and Managua and the Gulf of Fonseca that are at or near sea level. This depression is a rift structure associated with extension and subsidence (Phipps Morgan *et al.*, 2008; Funk *et al.*, 2009). In fact, the distribution of elevated beach lines above the present lake levels indicate that a broad inland seaway that connected these bodies of water existed as recently at 6,000 years before present (Roberto Protti, personal communication, January 2009). The arc volcanoes of Nicaragua are also among the lowest along the main Central American volcanic arc. On a more local scale, valleys between volcanic mountains can be many hundreds of meters lower in elevation than their peaks, as they are in the Central Volcanic Range in Costa Rica. It is not known if these

inter-volcano valleys are effective in filtering orchid gene flow. The possible impacts of regional lowlands will be discussed after interpreting distributions of taxa of Maxillariinae by country.

Speciation—Speciation is dominantly a biological process but one that can be influenced by environmental factors. Mutagenesis, the production of new biological forms with inheritable traits, is caused by damage to the nucleotide sequence of organisms and is affected by exposure to chemicals, high-energy radiation from natural decay products of radiogenic nuclides in nature, ultraviolet solar radiation, and cosmic rays from space. Mutations provide the inheritable variability that, when acted upon by geographic, environmental or sexual isolation, can lead to new species that do not cross with their forebears or closely related kin (Dodson and Gillespie, 1967). Mountain building clearly can cause geographic and environmental isolation.

It is often assumed that exposures to mutagens are random (and associated with normal biological processes such as cell division), that rates of genetic change are essentially constant, and hence that branching of the tree of life can be accurately dated by this genetic molecular clock. However, exposures to such mutagens can be spatially non-random. Ultraviolet and cosmic radiation have higher fluxes at higher elevations due to less screening by thinner atmospheres. Volcanoes produce a toxic brew of chemicals in gaseous form (SO₂, CO₂, HCl, HF, and radon) that can also interact in the atmosphere to produce sulfuric, carbonic, hydrochloric, and hydrofluoric acids, and other chemically aggressive species; these chemicals can kill plants and create dangers to human health. It is not known if sub-lethal exposures of these chemicals can induce mutagenesis in plants, a question that could be answered by experiment. Similarly, rocks vary widely in the levels of radioactive elements in their minerals, such as uranium, thorium, potassium (K40), and radon. Some granites and volcanic rocks found in mountain ranges have high enough radioactivity to pose potential health risks with long exposure. These examples might indicate that mutagenesis may not be spatially random and could occur at higher rates in tropical highlands. This possibility should be investigated in long-term experiments. Putting the question another way, such experiments would answer the interesting question:

Does the DNA molecular clock always run true (at a uniform rate) or does its going rate vary geographically and faster in some mountain ranges?

Extinction—As natural agents of destruction of habitats and life, volcanoes and volcanic eruptions are difficult to match in scale and within the spectrum of destructive volcanic processes (Baxter, 2000). Effusive volcanic eruptions are those involving non-explosive extrusions of magma to form lava flows, lava domes, and flood basalts. Lava flows are guided by topographic lows and can fill valleys and rivers, destroying whole riparian ecosystems. Dodson (2003) described such an event in Ecuador and its effects on valley orchid populations. Flood basalts can cover enormous areas, from hundreds to half a million square kilometers (Mahoney and Coffin, 1997). Unquestionably, events in this size range have caused biotic extinctions of species endemic to those areas and have probably been effective in interrupting orchid dispersal in the geologic past.

Giant explosive volcanic eruptions (sometimes called ‘super eruptions’) can have much more widespread and manifold effects on conditions for life on Earth (Mason *et al.*, 2004). Such eruptions can launch columns of hot tephra (ash) and gas as high as 50 km into the stratosphere. Volcanic aerosols suspended high in the atmosphere after the largest explosive eruptions have significantly cooled the planet for periods of months to years after the causative eruption. Global-scale volcanic crises are well documented in ice cores drilled from ice sheets in Greenland and Antarctica. Sequences of such eruptions may have cooled Earth for longer periods of time and hence had global climate impacts that are likely to have led to some extinctions of life forms due to multi-year cooling. Luckily, ‘super’ eruptions of this size have not occurred during historical times. However, the geologic record is marked by evidence for many giant explosive eruptions in Cenozoic time (the past ~65 million years). This evidence includes the formation of large volcanic calderas, features caused by large eruptions of magma associated with large-volume eruptions that lead to collapse of near-surface crust. Dozens of late-Cenozoic calderas with diameters greater than 5 km have been recognized in tropical Latin America. Another geologic indicator of the scope of explosive eruptions is in the distribution of ignimbrites (ash-flow sheets) that are products of collapse of the

hot eruption plume, a phenomenon called base surge, leading to pyroclastic flows of hot gas and tephra from the vent that can blanket areas as great as hundreds to tens of thousands of square kilometers and rock volumes of hundreds of cubic kilometers. Large ignimbrite fields of late Cenozoic age are common in Latin America, and such events probably led to destruction of large areas of forest cover and likely many regional extinctions of narrowly endemic orchids in the past. Repopulation of forests in the tropics can be fairly rapid given the high rainfall and the fertility of volcanic soils. In fact, Anak Krakatau, the island in the Sunda Strait left after the cataclysmic 1883 eruption in Indonesia, now has a dense tropical forest in place (Simkin and Fiske, 1983).

Valley-filling lahars (volcanic mud flows) can devastate lowlands and valleys near volcanoes and probably caused extinctions of narrow endemics in valley habitats in the American tropics similar to the effects of valley-filling lava flows and pyroclastic flows. Volcanic gases that boil out of magmas as they ascend to the surface during eruptions interact with atmospheric water to make acid rain and VOG (a ground-hugging volcanic fog) downwind from eruptive centers. Under certain conditions during prolonged eruptions, VOG can drift hundreds of kilometers from its source volcanic vent; volcanic phenomena have affected human health and have led to forest and cropland destruction. Moreover, volcanic eruptions can provide vital scientific information: they can be accurately dated from the isotopic makeup of the radioactive elements in some minerals that comprise volcanic rocks. These dates, in turn, can tell us when possible extinction events occurred or when geographic separation might have taken place between orchid populations. Chronologies of environmental events such as these may help establish absolute time marks on molecular clocks.

Young geological history of Costa Rica and Panama

The mountains in Costa Rica and Panama are, along with those in western Colombia and certain cordilleras in Ecuador and Peru, among the youngest in tropical Latin America. This condition is largely a consequence of the nexus of six moving tectonic plates and five trenches (where oceanic plates dive into Earth's mantle) in that region, and two major volcanic ridges (Cocos and Carnegie Ridges) that originate at the Galapagos hotspot and recently began colliding with the Pacific margins

of Costa Rica, Panama, Colombia, and Ecuador (Fig. 5; Mann and Corrigan, 1990; Mann, 1995; Coates, 1997; Wallace, 1997; Denyer *et al.*, 2003; Harmon, 2005; Mann *et al.*, 2006; Sak *et al.*, 2009). In a recent review of the chronologies of the volcanic and tectonic mountain ranges in Costa Rica, Denyer and Alvarado (2007) documented the remarkably young ages of the major phases of mountain building of these cordilleras (Table 1, Fig. 6), ranging from the Guanacaste and Central Volcanic Ranges (< 0.5 Ma BP [million years before present]) and the Fila Costeña (Pacific Coast Range) at << 2 Ma BP, 5 Ma BP for Cordillera Tilarán, and 4-10 Ma BP for the main Talamanca Range that extends into Panama and forms the mountain backbone of both countries (sparsely distributed older igneous rocks in the Talamanca may represent the roots of earlier island-arc volcanoes). Recent research indicates that the Talamanca Range has its highest elevations in Costa Rica as a consequence of underthrusting in the former forearc basin rocks of the Fila Costeña *under* the Talamanca, a process that started no earlier than 2 Ma BP (Fisher *et al.*, 2004; Morell *et al.*, 2007; Steichler *et al.*, 2007; Donald Fisher, personal communication, October 2009; Sak *et al.*, 2009). Thus the highest mountain range in Costa Rica and Panama was probably uplifted to its present elevation during Quaternary time (recently redefined as younger than 2.6 Ma BP).

These cordilleras are among the youngest mountain ranges in the world and were largely built during the time when our own species in the genus *Homo* evolved. Costa Rica and Panama are the youngest products of continent and mountain building in tropical Latin America. This region was formerly an oceanic seaway that was dotted by an island arc (subduction volcanic island chain) as recently as late Cenozoic times (25 Ma) and evidently at least served as partial barriers to the earlier dispersal of plant and animal species, famous in the annals of biogeography for the later great faunal exchange that was in full force by the beginning of Quaternary time (2.6 Ma BP; Webb, 1997). The gradual closing of this seaway by sediment accumulation and mountain building between present-day Colombia and the rest of Central America began about 12 Ma BP and was completed about 3 to 4.2 Ma BP, based mainly on evidence from marine biogeography and salinity that were recorded in marine sediments that provided a chronology of ocean circulation through the seaway

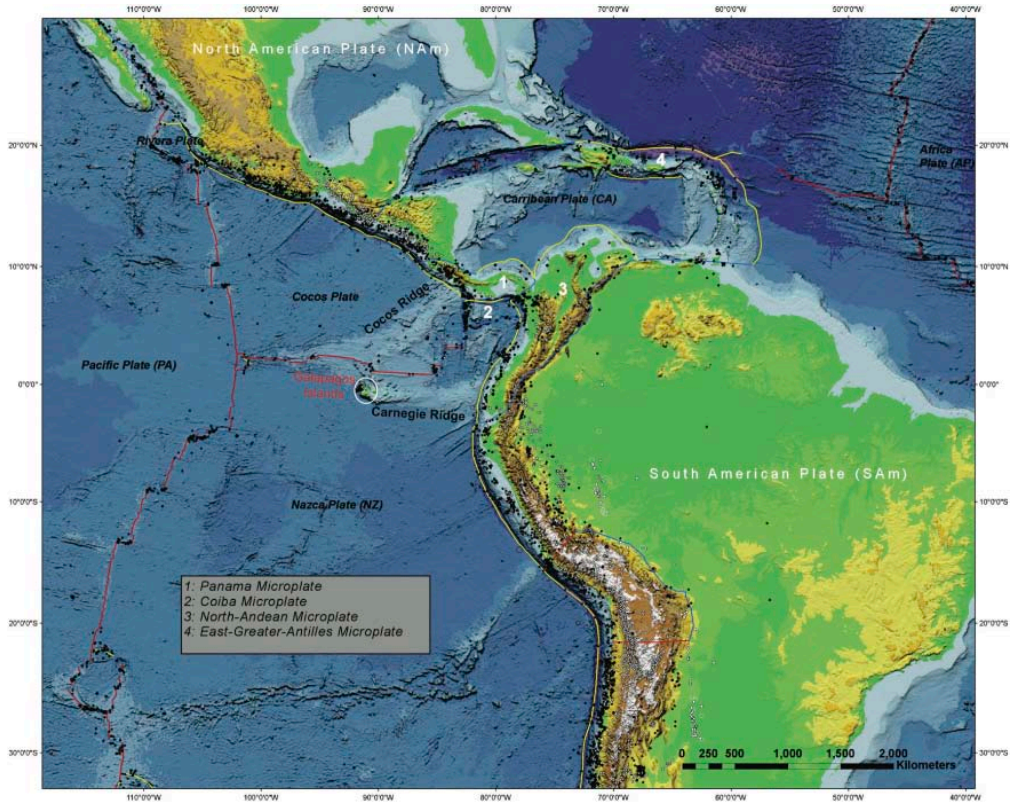


FIGURE 5. Plate-tectonics map of tropical America, including plate names and plate boundaries. Also shown are locations of volcanoes and volcanic centers (red triangles), earthquake epicenters (dots) and volcanic ridges originating at the Galapagos hotspot. This region is tectonically active, where landscapes and physical environments have been changing rapidly during Cenozoic time. Adapted from Simkin *et al.* (2006).

between the Atlantic-Ocean/Caribbean-Sea and the Pacific Ocean (e.g., Haug *et al.*, 2001).

Figure 2 shows the distribution of moist, tropical, broadleaf montane forests in Central America based on the Nature Conservancy “Map of the Eco Regions of Latin America.” I have highlighted on this map the windward fronts of mountain ranges, based on present-day prevailing wind patterns, that mark the northeast margins of moist, broadleaf montane forests in Central America and tropical Mexico. This map clearly shows that the total lengths and areas of such eco-regions in Mexico exceed those in Costa Rica and Panama. However, mountain building — tectonic and volcanic — in present-day tropical Mexico is far less active than in Costa Rica; the cordilleras in that part of tropical Mexico are far older than those in Costa Rica and Panama, and arc volcanoes are more sparse compared to the closely spaced volcanoes in the

continuous volcanic chain from Guatemala to southern Costa Rica. The central highlands of Honduras show similarities to those of southern Mexico. Geologically speaking, the rocks of the central Honduran highlands are pre-Cenozoic (older than about 65 Ma) and represent rocks accreted to the Central American isthmus over hundreds of millions of years (Rogers *et al.*, 2007). Instead of curvilinear cordilleras, most of these highlands are isolated mountains representing rock types that are resistant to erosion; many of these uplands are dominated by *Pinus* species. The present-day seismicity rate is also low in Honduras. Belize and Nicaragua support mostly lowland forest. Although Guatemala and El Salvador have active high volcanic cordilleras near their Pacific coasts, much of their forests are in lowlands. Finally, the Central American isthmus is narrowest in Costa Rica and Panama, and orographic effects of mountains tend to distribute

TABLE 1. Core Maxillariinae cladogram (Whitten *et al.*, 2007). Estimated total species numbers and established species counts in each genus from Blanco *et al.* (2007).

	Reassigned Genus	Clade Code	Estimated Total Species Numbers	No. Species Classified in Blanco <i>et al.</i> (2007)
99	<i>Maxillaria s.s.</i>	Q	200-250	163
100	<i>Camaridium</i>	P	80	72
63	<i>Trigonidium</i>	O	7	7
100	<i>Maxillariella</i>	N	50	47
100	<i>Rhizinantha</i>	M	15	15
100	<i>Mormolyca</i>	J	25	23
100	<i>Cyrtiorchis</i>	H	5	5
100	<i>Sarvetrea</i>	I	15	13
100	<i>Christensonella</i>	L	12	13
100	<i>Mapiquari</i>	K	4	5
100	<i>Cryptocentrum</i>	G	18	-
65	<i>Inti</i>	F	2	3
84	<i>Brasiliorchis</i>	E	13	13
93	<i>Pityphyllum</i>	D	7	7
100	<i>Ornithidium</i>	C	60	55
96	<i>Heterotaxis</i>	B	13	15
77	<i>Ninodolobon</i>	A	3	3
24				
79				
100	<i>SLM SPECIES</i>		554	

rainfall over a much narrower area of the highland isthmus than farther north.

The foregoing observations show that the present-day distribution of tropical cloud forest environments alone does not explain why so many more orchid species are endemic to the southernmost countries of Central America, where orchid diversity tends to be most exuberant. Of course, the latter are at lower latitude than tropical regions farther north, and perhaps higher average temperatures and greater rainfalls may be playing roles in these differences in orchid diversity. However, tradeoffs of warmer conditions at lower elevation ranges may negate the lower temperatures at higher latitudes. For many orchid tribes, centers of diversity are in South America, such that present-day orchid species distribution may be a snapshot of a general trend toward northward dispersal from these centers. In light of these observations, I consider two working hypotheses in this study: 1) hotter and drier lowland gaps between the cordilleras of Central America reduce the rates of this northward dispersal of species adapted to highland conditions (and possibly the southeasterly counter gene flow of species endemic to northern Central America), and 2) high rates of environmental change connected with mountain building in late Cenozoic times are significant factors in promoting more rapid orchid speciation in Costa Rica and Panama. These biologically important factors associated with mountain building are applied to the country distribution data for core Maxillariinae in Central America and southern Mexico.

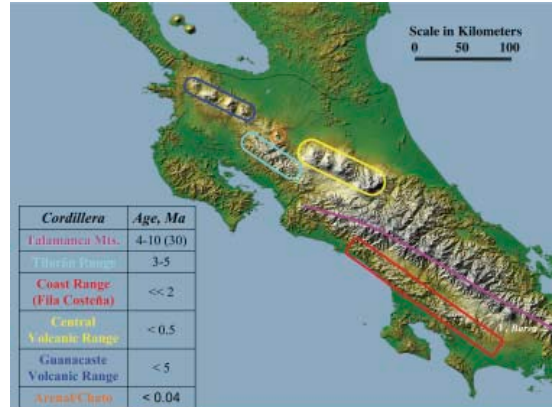


FIGURE 6. Color-shaded relief map of Costa Rica showing the locations of the principal mountain ranges, their approximate ages, and active and recently active volcanoes (inset). Based on Denyer *et al.* (2003), Denyer and Alvarado (2007), and information from Donald Fisher (Pennsylvania State University, personal communication, October 2009).

A case study: distribution of Maxillariinae in Southeast Mexico and Central America and adjoining regions

Data and methodology—To explore the above-mentioned working hypothesis, I have analyzed the distribution of species in core Maxillariinae in Central America. There are several reasons that these are good taxa to use. There is a large number of species (550- 580) according to Whitten *et al.* (2007) and Alrich and Higgins (2008). Most of these species are relatively large, conspicuous, and locally abundant. These species have diverse flower and plant traits and display a range of pollination syndromes. Ossenbach *et al.* (2007) documented 160 species in Central America and tropical Mexico (the states of Chiapas, Tabasco, Veracruz, and Puebla). Members of these taxa are found in all of the countries of South America and the Antilles over a wide range of elevations. Finally, this group of species has received the attention of a recent and extensive molecular phylogenetic study by Whitten *et al.* (2007) using multiple molecular markers, resulting in a well-supported generic realignment of the subtribe into 17 clades (Table 1). This was followed up by a reclassification of the subtribe by Blanco *et al.* (2007) based on the molecular data and phenotypical characters described by Whitten *et al.* (2007).

My starting point for country distribution data on species in core Maxillariinae are the checklists by Ossenbach *et al.* (2007) and Atwood (2003). I supplemented these resources with the following online herbarium data resources: *Tropicos* (Missouri Botanical Garden), INBIO, Lankester Botanical Gardens *Epidendra* database, the Costa Rica National Herbarium, and the *Worldwide Checklist of Monocotyledons*. To resolve questions of synonymy, I largely relied on the assessments in the online *Tropicos* checklist and papers by Atwood and Mora de Retana (1999) on the subtribe. For Central American nations, I also relied on the most recent orchid monographs by countries listed below in Literature Cited. For tropical Mexico, I also consulted Soto *et al.* (2007) and H.gater *et al.* (2005). In the interest of exploring distribution differences between Central and South America, I checked the distribution of species found in Central America and tropical Mexico that have also been collected in South America and the Antilles by consulting Misas-Urreta (2005) for near-coastal northwest Colombia; an unpublished database of herbarium collections by Dodson for Ecuador as well as his splendid five-volume book series (2000-2004); a checklist by Dodson of Colombian *Maxillaria* species in Ospina (1996); the four-volume book series *Native Colombian Orchids* edited by Escobar (1990) and Dodson (2002, 2003b); Zelenko and Bermudez (2009) for Peru; McLeish *et al.* (1995) for Belize; Dunsterville and Garay (1979) for Venezuela; and Nir (2000) for the Antilles. In cases of differences in distribution data between sources, my preference was generally to adopt distribution data for vouchered herbarium specimens that were collected and identified in recent decades. Many ambiguities exist in this distribution database, for which some of my choices may have been in some cases somewhat subjective and arbitrary. Elevation data for specimen collection were generally taken as those reported except where geographic locations were incompatible with the stated elevations. All of these summaries of the orchid flora suffer from varying degrees of undersampling. Of particular concern is undersampling in northwest Colombia and eastern Panama due to security challenges, a shortcoming that may bias assessments of the distribution data of species common to both Central and South America.

Country abbreviations in this study are as follows: Bolivia (Bo), Peru (Pe), Ecuador (Ec), Colombia (Co),

Panama (Pa), Costa Rica (CR), Nicaragua (N), El Salvador (ES), Honduras (H), Guatemala (Gu), Belize (Be), Mexico (Mx), Greater Antilles (GA, including southern Florida, USA), Lesser Antilles (LA), Guyana (Gy), and Venezuela (V).

Results

Geographic distribution—The country distribution data were classified according to the following scheme based on the distribution patterns that were evident (Table 2):

- Class 1: Species endemic to Costa Rica and/or Panama (Southeast Central America – SE CAM) [83 species in this class]
- Class 2: Species endemic to both SE CAM and at least two countries in northern Central America (N CAM) [Nicaragua, Honduras, Guatemala, ±Mexico ±Belize and ±El Salvador] [13 species]
- Class 3: Species endemic to both SE CAM and northwest South America (Colombia, Venezuela, Ecuador, Peru) [31 species]
- Class 4: Species endemic to N CAM [9 species]
- Class 5: Pan Latin America (N CAM and SE CAM and NW S Am) [18 species]
- Class 6: Occurrence in Ossenbach *et al.* (2007) not verified in CAM in this study but reportedly found in SAM [6 species].

TABLE 2. Classification scheme of country distribution of core Maxillariinae species reported in Central America according to their north-south geographic spread by country in tropical Latin America. Notes: ± = may or may not be present; * Distribution Code 1: Pa and/or CR; Code 2: Pa and/or CR + two or more NW CAM countries; Code 3: Pa and/or CR + 2 or more of Co, Ec, or Pe; Code 4: 2 or more N, Gu, or Mx; Code 5: SE CAM + 2 or more NW CAM + 2 or more NW CAM.

Code*	South					North					Distribution	No. Species		
	Bo	Pe	Ec	Co	Pa	CR	N	ES	H	Gu			Be	Mx
1					✓	✓							Panama – CR Endemics ± Ni (SE CAM)	83
2					✓	✓	✓	*	*	✓	*	✓	CAM Endemics	13
3	±	✓	✓	✓	✓	✓							SW CAM + SAM Endemics	31
4							✓	*	*	✓	*	✓	NW CAM Endemics	9
5	✓	✓	✓	✓	✓	✓	✓	*	*	✓	*	✓	Pan-Tropical American Endemics	18
6	South American endemics or uncertain distribution in the Ossenbach <i>et al.</i> (2007) checklist											6		

Increasingly Restricted Endemism ↑

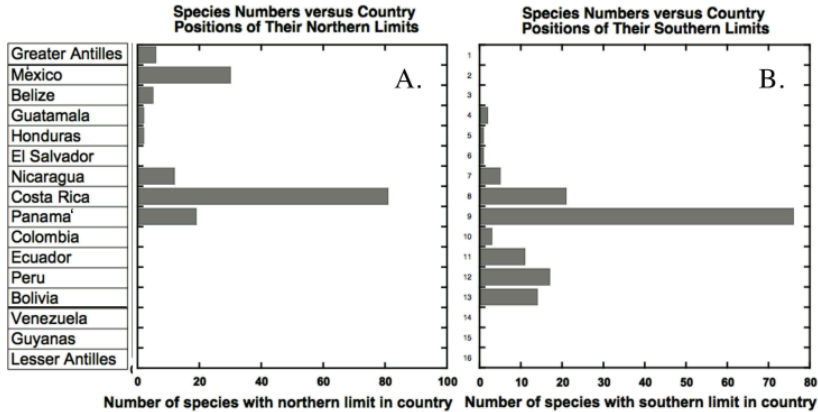


FIGURE 7. Histograms showing the northern and southern limits of geographic ranges of core Maxillariinae species occurring in Central America. A. Northern limits by country. B. Southern limits by country.

Several observations may be made concerning species levels in these geographic classes. First, Costa Rica and Panama have by far the greatest number of endemic species among Central American nations and tropical Mexico, a finding that mirrors the overall level of orchid species endemics among these nations and one that has been recognized earlier by others based on more limited data. However, as discussed below, this result is weighted heavily in just a few clades recognized in the Whitten *et al.* (2007) study. Second, more species in Maxillariinae in Costa Rica and Panama have species in common with South America (31) than other countries in Central America and tropical Mexico (13). Combined with the fact that nearly three-quarters of all species in Maxillariinae are found only in South America indicates that the primary ancestral evolutionary center for the subtribe was on that continent, with a secondary center in Costa Rica and Panama and with lesser endemism in northern Central America, tropical Mexico, and the Antilles.

It is also useful to examine the northern and southern geographic limits by country of species in this taxa for species that occur in Central America and tropical Mexico (Fig. 7). By far, the largest number of species have northern limits in Costa Rica (Fig. 7A) and southern limits in Panama (Fig. 7B). This finding follows from the high number of endemics in these two countries. Thirty species have northern limits in Mexico, perhaps reflecting a somewhat lower total number of Mexican endemics (Fig. 7A), the northern limits of tropical climate, and also the longer potential

dispersal distance to the Greater Antilles and southern Florida. Few species in this tribe have northern or southern limits in Belize, Guatemala, El Salvador or Honduras.

Elevational distribution — I show the distribution of species in Maxillariinae with elevation range in Figures 8 and 9 for two of the six distribution classes studied. Class 1 species (83 total), those with the narrowest distribution, tend to have elevation limits of 500 m or above; these are clearly species (74 in number) mostly adapted to the cooler and wetter conditions found in the cloud-forest highlands of Costa Rica and Panama (Fig. 8A, 9A). There are nine exceptions to this trend: species that are restricted to Costa Rica and/or Panama and also have their lower elevation limits below 200 m (*Camaridium suaveolens* (Barringer) M.A.Blanco, *Maxillariella diuturna* (Ames & C.Schweinf.) M.A.Blanco, *Mormolyca dressleriana* (Carnevali & J.T.Atwood) M.A.Blanco, *Maxillaria endresii* Rchb.f., *Ornithidium nicaraguense* (Hamer & Garay) M.A.Blanco & Ojeda, *Maxillariella oreocharis* (Schltr.) M.A.Blanco & Carnevali, *Camaridium latifolium* Schltr., *Maxillariella sanguinea* (Rolfe) M.A. Blanco & Carnevali, and *Camaridium vittariifolium* (L.O.Williams) M.A.Blanco according to the realignments by Whitten *et al.* (2007) and Blanco *et al.* (2007). Finally, no species in this geographic distribution class shows an elevation range greater than about 2000 m above their lowest reported elevation, an interesting limitation that may have a physiological

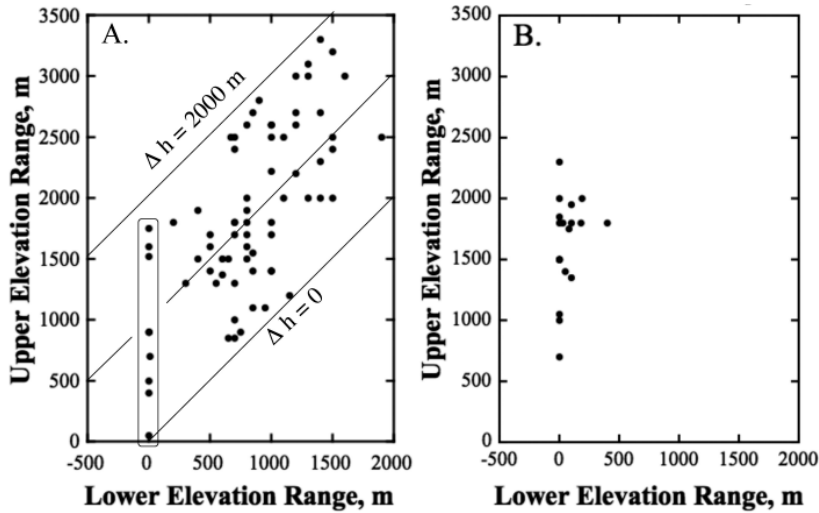


FIGURE 8. Elevation ranges for core Maxillariinae species plotted as lower elevation limit on the horizontal axis versus upper elevation limit on the vertical axis. A. Geographic Class 1 species (Costa Rica and/or Panama endemics). Δh is the elevation range. Note that most of these species have lower elevation limits greater than 500 m. Species that are exceptions enclosed by the vertical box are listed in the text. B. Class 5 species (Pan Latin American species, all lowland).

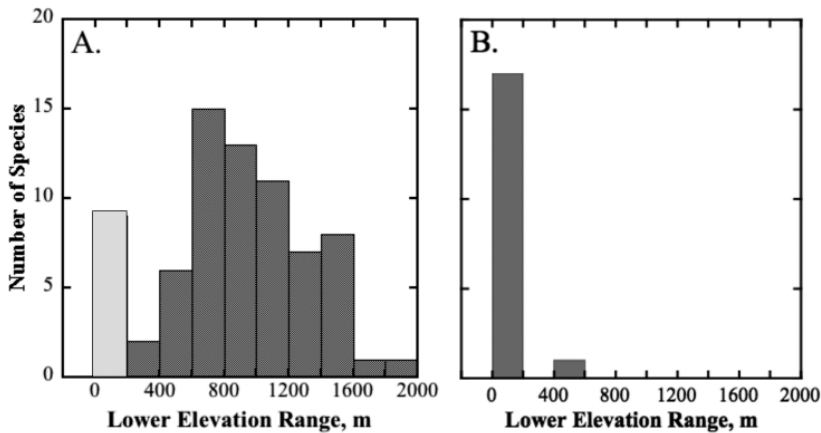


FIGURE 9. Comparisons between the histograms of the lower elevation limits for core Maxillariinae species for two different geographic distributions. A. Class 1 species (Costa Rica and/or Panama endemics), largely upland species with exceptions noted in text (light gray) B. Class 5 species: Pan Latin American species, all extending to low elevations.

origin. For Class 5 species, the Pan Latin American ones, all 18 have lower elevation limits below 500 and most (17) below 200 m (Fig. 8B, 9B). The wide geographic distribution of this class indicates that part of their successful dispersal may be rooted in their evident adaptation to lowland conditions and hence easier dispersal than those adapted to exclusively higher elevation ranges and likely subject to strong dispersal ‘filters’ across generally hot, dry lowland gaps between cordilleras.

Geographic distribution of clades—Table 3 summarizes the distribution of the clades of Whitten *et al.* (2007) among the geographic distribution classes identified in this study. Summing the species numbers for classes 1 through 5 shows that *Camaridium* is easily the most abundant genus of core Maxillariinae in Central America, representing 62 of the total of 72 species that Blanco *et al.* (2007) recognized in this genus. This important genus of tropical epiphytes represents about 15% of all species in the core Maxillariinae and

TABLE 3. Geographic distribution of species in core Maxillariinae following Whitten *et al.* (2007) and Blanco *et al.* (2007). See notes in text and at bottom of this table.

Clade	Clade/Genus Name	1	2	3	4	5	Sum 1 thru 5	All Latin America	% Species in Central America
Code [^]	Geographic Classes→	SE CAm = CR + Pa	Pan CAm = N & SE CAm	SE CAm + SAm	N CAm	Pan LAm		Blanco <i>et al.</i> (2007)*	
A	<i>Nitidobulbon</i>					1	1	3	33
B	<i>Heterotaxis</i>		1			3	4	15	27
C	<i>Ornithidium</i>	4		1	1	1	7	55	13
D	<i>Pityphyllum</i>						0	7	0
E	<i>Brasiliorchis</i>						0	13	0
F	<i>Inti</i>			2			2	2	100
G	<i>Cryptocentrum</i>	3		4			7	18	39
H	<i>Cyrtidiorchis</i>						0	5	0
I	<i>Sauvetea</i>	1					1	13	8
J	<i>Mormolyca</i>	1	2			1	4	23	17
K	<i>Mapinguari</i>			1			1	5	20
L	<i>Christensonella</i>					1	1	13	8
M	<i>Rhetinantha</i>	1	1			2	4	15	27
N	<i>Maxillariella</i>	5	3	2	2	3	15	47	32
O	<i>Trigonidium</i>	1		2		1	4	7	57
P	<i>Camaridium</i>	52	3	3	1	3	62	72	86
Q	<i>Maxillaria s.s.</i>	11	2	11	4	1	29	163	18
	SUMS →	79	12	26	8	17	142	476	30
[^] As coded by Whitten <i>et al.</i> (2007)									
[@] CAm means Central America including southern Mexico; SAm means South America; Pan means throughout; Latin Am means Central and South America; SE means southeastern; N means northern. See Table 1.									
[#] Only species classified by clade in Blanco <i>et al.</i> (2007) are included in this sorting. Differences with Table 1 in the number of species within each genus are due to a number of species being suspected of being within certain genera (Table 1), but not yet proven.									
[*] My count of species placed by Blanco <i>et al.</i> (2007) in these genera, not their estimated total number.									

represents a floristically and vegetatively diverse group that ranges from large multifloral cane-like species such as *C. biolleyi* (Schltr.) Schltr., *C. bradeorum* Schltr., and *C. inauditum* (Rchb.f.) M.A. Blanco to the striped miniatures *C. wercklei* Schltr. and *C. tigrinum* (C. Schweinf.) M.A. Blanco to the challenging *C. cucullatum* (Lindl.) M.A. Blanco complex. About 72% of *Camaridium* species are endemic to Costa Rica and Panama (Table 3), and only three are exclusively shared with South America and three with the rest of Central America. Only three *Camaridium* species are reported to be endemic to other Central American countries, and another three have a Pan Latin American distribution. It is therefore a reasonable inference that this genus evolved in the Costa Rica/Panama region.

Since species in this genus largely occur in highland cloud-forest environments, most of these speciation events could not have predated the mountains in which they are endemic, that is, late Cenozoic time (mostly Pliocene and Quaternary or the last 5 million years to 500,000 years depending on the mountain range). Only limited dispersal of this genus to other Latin American countries has evidently occurred since then.

Except for two genera with a small number of species (*Inti* and *Trigonidium*), Central American endemic species in other genera in core Maxillariinae, as defined by Whitten *et al.* (2007) and listed in Blanco *et al.* (2007), represent minority populations compared to species in those genera in South America (Table 3). Notable among these genera are *Maxillaria sensu stricto* (only 18% occur in Central America out of a total of 165 species placed in that genus by Blanco *et al.* (2007), the largest clade in the subtribe), *Maxillariella* (32% of 50 species), *Mormolyca* (16% of 25 species), *Heterotaxis* (31% of 13 species), *Sauvetea* (8% of 13 species), and *Ornithidium* (17% of 60 species). Since the geographic centroids of species in these genera are clearly in South America, it is plausible that they originated there. However, this low representation in Central America may be partly a consequence of the smaller land areas of Central American countries compared to South America. The balance of the 17 genera of Whitten *et al.* (2007) either have few species and/or have few (if any) species in Central America.

The genus *Ornithidium* has an estimated 60 species, all Neotropical. Among the seven *Ornithidium* species

reported in Central America, four are highland species (*O. hagsaterianum* (Soto Arenas) Senghas, *O. conduplicatum* Ames & C.Schweinf., *O. pittieri* Ames, and *O. repens* (L.O.Williams) M.A.Blanco & Ojeda), and three are reported to have distribution ranges extending down to lowland forests (*O. nicaraguense* Hamer & Garay) M.A.Blanco & Ojeda, *O. adendrobium* (Rchb.f.) M.A.Blanco & Ojeda, and *O. fulgens* Rchb.f.). *Ornithidium fulgens*, reported from Bolivia to Mexico and also in Venezuela, is known to be frequently visited and presumably pollinated by hummingbirds (Fogden and Fogden, 2006) and is the most widespread of the genus. A number of species in this clade resemble *O. fulgens* (small, globose or partially closed flowers; bright red, pink or yellow with reflexed sepals and petals; bright yellow, red, or yellow/red, fleshy, and often saccate lips, some reported to produce nectar). These species include: *O. aggregatum* Rchb.f., *O. aureum* Poepp. & Endl., *O. canarense* (J.T.Atwood) M.A.Blanco & Ojeda, *O. coccineum* (Jacq.) Salisb. ex R.Br. (the type species for the genus *Ornithidium*), *O. conduplicatum* Ames & C.Schweinf., *O. giganteum* Lindl., *O. jamesonii* Rchb.f., *O. miniatum* Lindl., *O. pittieri* Ames, *O. ruberrimum* (Lindl.) Rchb.f., and *O. semiscabrum* Lindl. All for which we have elevation data occur in highland forests where hummingbirds are said to have a selective advantage over insects in being able to feed at low temperatures (van de Pijl and Dodson, 1966; van der Cingel, 2001). However, *O. fulgens* is geographically and elevationally widely dispersed, in spite of the presence of many lowland gaps in which hummingbirds do not have such a selective advantage, but to which it is nonetheless evidently well adapted.

Discussion

The foregoing general observations and interpretations lack specifics regarding precise information on the geographic and elevation distribution of individual species, a limitation that accompanies the exclusive use of country distribution data in this study. More distribution information is now becoming increasingly available online, and collection locations are established using GPS receivers. These advances will enable the use of Geographic Information Systems (GIS) to plot distributions on map bases using advanced satellite-based topographic data,

such as the SRTM data (Shuttle Radar Topographic Mission). A recent GIS study by Lorena Endara of orchid distribution in Ecuador shows the power of such methods (Endara *et al.*, 2009). Such methods may also enable the investigator to cross-check geographic data with elevation data and use cultural location information (e.g., state and nearby town and river names) often recorded in older reports. Also, climate and forest cover information may also be compared with orchid species distribution data. Some botanical institutions are already employing these methods.

Another limitation of virtually all sampling is that collections are often conducted in the campaign mode, *i.e.*, go out and collect for a few days or weeks and then return to study your plants and flowers. These collections are valuable but potentially suffer from undersampling because of possibly collecting outside the flowering periods of some species and the small number of forest trees or terrestrial environments actually sampled. The writer is co-founder of the Bosque de Paz Orchid Survey, a 2000-hectare biological reserve located in the upper Río Toro Valley in Alajuela Province in Costa Rica and now in its 7th year. Orchid collections from downed trees and tree limbs are made during maintenance of such as this. Such studies, however, are fairly costly, time-consuming, and rare.

Finally, the density of sampling for molecular phylogenetics has generally not progressed down to individual species circumscriptions (or closely related species) such that vegetative and floral traits are useful in establishing clues as to dispersion pathways or geographic separations. As orchid genotyping becomes less expensive, and more and more individual plants per species are sequenced, subtle differences in genetic markers may give more clues to these pathways than species distributions alone.

Conclusions

In a noteworthy recent biography of Charles Darwin, Quammen (2007), author of the immensely popular and readable book about island animal biogeography, *the Song of the Dodo*, makes an important observation that just as Copernicus (1473-1543) put the sun, rather than planet Earth, at the center of our solar system, Darwin identified a process that placed *Homo sapiens* as just a mammalian species in a long succession of

life forms shaped by natural selection. One can take this concept another step. As a geologist in an era of great strides in increasing understanding of our planet Earth, Darwin also helped put our time, our geography, and our climate into a chronology of a long succession of environmental changes that were shaped in part by geological processes. He therefore not only helped enlarge our view of the true time span of life on Earth that enables natural selection to work, but his geological investigations also helped point to how such changes can be drivers for evolution.

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THE ROLE OF COMMON ORCHIDS IN APPRECIATING THE COMPLEXITY OF BIODIVERSITY CONSERVATION

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ABSTRACT. To conserve a species, we must understand its biology, ecology, and relative vulnerability to change. To conserve biodiversity, we need a profound understanding of the relative impact of natural and anthropogenic disturbances and species interactions and of the role of the ecosystem in species survival. Assumptions of the basis for abundance and decline and therefore conservation status might be ill founded if we miss critical aspects of life history or of inter-relationships with other organisms. For more than two decades we have monitored populations of two common terrestrial orchids that grow in close proximity in Gatineau Park, Québec, Canada. The relative abundance and availability of large populations of *Cypripedium parviflorum* var. *pubescens* and of the introduced *Epipactis helleborine* presented us with an opportunity to conduct in situ experimentation, and to develop and evaluate conservation approaches not possible with uncommon or rare orchids. We have learned that the distribution, patchiness, and persistence of *E. helleborine* is related to the presence and diversity of trees and that seeds of this orchid in trampled soil may germinate better than those in undisturbed soil but that location is a likely overriding variable. Experimental trampling within colonies of common orchids has revealed that the fungivorous nematode community is negatively impacted by foot traffic, which could be reflecting subtle changes in the soil fungal assemblage upon which the nematodes feed. While mature plants of *C. parviflorum* var. *pubescens* do not seem to have been affected by nearby foot traffic, seed germination and seedling survival could have been altered, but we may not become aware of such changes for some time.

RESUMEN. Para conservar especies se debe comprender su biología, ecología y su relativa vulnerabilidad a cambios. Para conservar la biodiversidad, necesitamos un entendimiento profundo del impacto de los disturbios naturales y antropogénicos, de las interacciones y, del rol que juega el ecosistema en la supervivencia de las especies. Las suposiciones de abundancia y declinación y por consiguiente el estatus de conservación puede ser totalmente erróneo si no se toman en cuenta aspectos críticos de la historia natural o las relaciones con otros organismos. Por más de dos décadas hemos monitoreado las poblaciones de dos orquídeas terrestres que crecen en la cercanía del Parque Gatineau, Québec, Canadá. La abundancia relativa y disponibilidad de poblaciones grandes de *Cypripedium parviflorum* var. *pubescens* y de la especie introducida *Epipactis helleborine* nos dio la oportunidad de realizar experimentos in situ y de desarrollar y evaluar aspectos de conservación los cuales no son ejecutables con orquídeas poco comunes o raras. Hemos aprendido que la distribución, congregación y persistencia de *E. helleborine* está relacionada con la de diversidad de árboles y también que las semillas de las orquídeas germinan mejor en tierra apisonada que en aquella que no presenta disturbio, sin embargo, la localidad sería una variable preponderante. Apisonamiento experimental ocasionado por el tráfico de transeúntes dentro de las localidades de colonias de orquídeas comunes ha revelado un impacto negativo en las comunidades de nemátodos fungívoros, lo que podría reflejar ligeros cambios en el ensamblaje fúngico del cual los nemátodos se alimentan. Entre tanto, las plantas maduras de *C. parviflorum* var. *pubescens* no parecen ser afectadas por los efectos que tienen los transeúntes en el sendero. Sin embargo, la germinación y la supervivencia de las plántulas podrían haber sido alteradas, pero no nos daremos cuenta de estos cambios en algún tiempo.

KEY WORDS: Orchidaceae, conservation, disturbance, trampling, *Cypripedium parviflorum* var. *pubescens*, *Epipactis helleborine*

When we first considered monitoring terrestrial orchid populations in Gatineau Park, Québec, Canada, one of our concerns was the potential impact of our visits on rare or uncommon species and their habitats. We chose instead to monitor populations of two very common orchids, *Cypripedium parviflorum* var. *pubescens* (Willd.) O.W.Knight and the introduced European orchid, *Epipactis helleborine* (L.) Crantz. Not only were there large populations consisting of hundreds of individuals but there was the additional possibility to conduct *in situ* experimentation which might not have been possible with rare species and certainly not on a large scale. We felt that our observations might be of future benefit to conservationists working with rare taxa where the outcome of interventions can be fraught with uncertainty. To minimize impact on orchid habitat, we followed a set path within sites and limited the number of visits to the minimum needed to obtain data. Despite these precautions, we realized that there would be some disturbance (however minimal) and that annual monitoring could have an unpredictable impact on orchid survival in the research sites. We wondered how we could assess our physical impact on things we could not see or predict and what measures could be undertaken to mitigate further monitoring impact on the orchid ecosystem. In 2006, we began a study to quantify visitor impact on orchid habitat. *Cypripedium parviflorum* var. *pubescens* was selected for this study because it was shallow-rooted and therefore potentially more vulnerable to trampling (Light & MacConaill, 2007). We employed a daily 5-minute standing visit in pre-set locations for 10 days during the orchid blooming season to simulate a typical observer visit. Trampling spots were located where one might stand to count flowers or take photographs, or within 50 cm of a flowering plant. We wanted to quantify soil compaction, characterize the impact of trampling on soil organisms, and track recovery from trampling. Soil nematodes were selected as a bioindicator because they have been demonstrated to be an excellent indicator of soil health and can also be easily extracted and identified to trophic or feeding group based upon mouth and gut structure (Neher, 2001). This first study, with follow-up measurements during 2007, demonstrated that 10 daily standing visits compacted soil and perturbed the nematode community. These changes were still significantly different from controls

after 16 months (Light & MacConaill, 2007, 2008). We needed to learn how long the trampling effect of this first experiment would endure, if the results could be repeated in different habitats, and if trampling modified orchid seed germination behavior. We decided to use the seeds of *E. helleborine* instead of the *Cypripedium* for this first assessment of trampling impact on seed germination because we already had conducted a preliminary study of *in situ* germination of *E. helleborine*.

Materials and methods

Effects of visitor trampling on a terrestrial orchid habitat — In 2008, the earlier trampling experiment (Light & MacConaill, 2007) was repeated in three nearby yet different sites in Gatineau Park, Québec, Canada, that had the following characteristics. Site A was a long-term study site located in open forest (Light & MacConaill, 2002 a,b): clay soil, pH 6.4 (range: 5.8–7.0; n=15); dominant trees, *Acer saccharum* Marshall and *Quercus rubra* L.; orchid, *Cypripedium parviflorum* var. *pubescens*. Test and control plots were placed near previously mapped orchid plants with foot-traffic history (plots were located where no visitor traffic was likely to have occurred over the past 10 years). Site S was an isolated forest clearing located about 150 m to the south of A: clay soil, pH 6.4 (range: 6.1–6.8; n=15); dominant tree, *Acer saccharum*; orchid, *C. parviflorum* var. *pubescens*. Site H was a small valley located about 50 m to the north of Site A featuring a centrally located game trail: humus soil with low clay content, pH 6.5; range, 6.1–6.8, n = 15; dominant trees, *Quercus rubra*, *Pinus strobus* L., and *Acer saccharum*. Site H had previously supported a small population of *Platanthera hookeri* (Torr.) Lindl. We established experimental plots along either side of the valley off the game trail and away from these orchids.

Experimental trampling consisted of a 5-minute standing visit daily for 10 consecutive days during the flowering period of *C. parviflorum* var. *pubescens* in May. Soil compaction and temperature were assessed daily for a total of 30 days before, during, and after the experimental trampling period, and monthly thereafter until September. A pocket penetrometer (Cole-Palmer) was used to measure resistance to penetration of the soil by a standard cylinder. The nematode community

composition of all experimental plots and the associated research trails was assessed as previously described (Light & MacConaill, 2007), with paired soil cores (5.5 cm dia x 3 cm deep) being taken from a randomly selected footprint in each sample plot in the first year and from the opposite footprint in the second year. Insufficient area remained for coring of footprints in the third year (2008), although sufficient footprint surface was still present for the compaction measurements in those plots.

Distribution and association of Epipactis helleborine with large trees—*Epipactis helleborine* is locally common in Gatineau Park where we have extensive knowledge of its biology and distribution in our long-term study Site 1. It was in this site that we noticed a clear association with a large tree, 44 cm diameter at breast height (dbh), rather than with any of the 31 much smaller trees and saplings (1 to 11.5 cm dbh) that shared the site (Light & MacConaill, 2006a). The 15 largest trees in an area of open forest adjacent to this site were identified and mapped during the autumn of 2005 (Fig. 1). These trees were 15 to 20 m tall and ranged in diameter (dbh) from 17 to 75 cm with a median value of 45 cm. The scattered smaller trees and saplings in this area were mainly *Ostrya virginiana* (Mill.) K.Koch. All *E. helleborine* plants emerging during 2006 within a 3 m radius from the trunk of each of the 15 large trees (total area approx. 445 m²) were counted and mapped. For the purpose of comparison, we surveyed a similar-sized, sparsely forested area outside those circles (Fig. 1). All orchids emerging in this area were counted and mapped. Trampling during this survey was minimized by walking whenever possible on exposed rocks and by keeping to an established assessment trail. Plant densities in selected areas were compared by calculating the χ^2 statistic (Sokal & Rohlf, 1981) for the differences between the observed numbers in those areas and the numbers expected from the null hypothesis of a uniform distribution across the total relevant area.

Impact of trampling on germination of Epipactis helleborine—We used our knowledge of *E. helleborine* distribution from the 2006 survey to place seed packets where we expected to obtain the most useful results: where orchids were growing and mycorrhizal fungi were likely present, within 5 m of a large tree. Mature

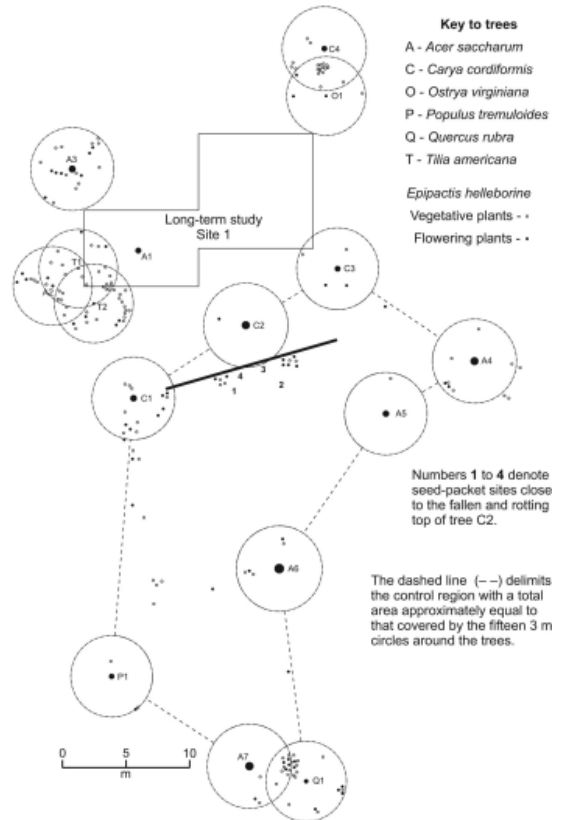


FIGURE 1. Location of 15 large trees, vegetative and flowering *E. helleborine* found in 2006, and seed packet burials (1 to 4) within 5 m of a dead *Carya cordiformis* (C2) and its fallen treetop (solid line). Trees are circumscribed by 3 m radius circles.

fruits were harvested at the time of dehiscence from large plants growing in the same general vicinity as the experiment. Seeds were dried and stored at room temperature for 60 days over silica gel until the day before preparation and placement of seed packets (November 1, 2006). Seed packets were constructed of fine nylon mesh enclosed in 35 mm plastic slide mounts. Approximately 150 to 200 seeds were placed in each packet and the mounts stapled at three points. A small hole was punched through one corner of a mount through which a length of nylon fishing line was fastened. All packets could thus be tethered to a central plastic peg to simplify retrieval. Four groupings of 3 packet-pairs per plot (24 seed packets in all) were buried within 5 m of a large tree, *Carya cordiformis* (Wangenh.) K.Koch, that had died after a 1998 storm had broken

the treetop (Fig. 1 – C2). The fallen treetop log formed the upper boundary of packet burials (Fig. 1). Pairs of tethered seed packets were buried in groups of 3 with pairs arranged at 120-degree intervals around a central peg. Two groups were sited 1 m apart and about 50 cm from the log. The two other groups were sited about 1 m apart and away from the others. The packet groups were located between the two clusters of *E. helleborine* plants that had emerged in 2006 (Fig. 1). To minimize soil disturbance during packet placement, a stout knife was used to cut slots in the soil into which packets were placed horizontally about 5 cm beneath the surface. For each group of 3 packet-pairs, two pairs were randomly chosen for trampling at a rate of 30 footsteps per packet (16 packets trampled in all). Controls were untrampled. Soil resistance to penetration was assessed before and after packet placement and two years later just before the packets were removed (November 2008). Packets were washed, opened and scored for germination and seedling development (Stage 1 - swollen embryo–testa not split; Stage 2 - swollen embryo–testa split; Stage 3 - protocorm with rhizoids; Stage 4+ - protocorm with root(s)).

Results

Follow up of the 2006 study—Residual soil compaction still persisted 28 months after the initial 2006 study (Fig. 2). Soil compaction measurements still tracked with soil temperature (Fig. 2), with resistance to penetration increasing and decreasing concomitantly with soil temperature. Both trampled and untrampled soils behaved similarly. Because we had already removed four cores from each pair of footprints, there was insufficient surface area remaining for core sampling of trampled plots in the third year. There was, however, sufficient trampled surface remaining to measure residual compaction.

Effects of trampling in different habitats—The results were essentially the same at each of the 3 sites. Soil compaction increased with each trampling episode during the 10- day experimental period and remained significantly higher than controls in each of the sites for the four months after experimental trampling until the experiment was terminated (Fig. 3). Heavy rainfall on two occasions induced a brief soil softening before recovery.

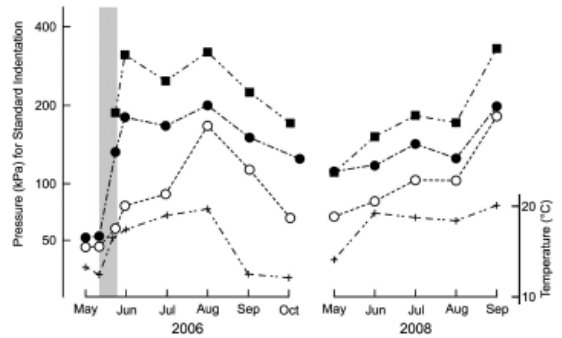


FIGURE 2. Soil compaction recovery over 28 months following 10 days of daily 5-minute standing visits in May 2006 (2007 data omitted for brevity). Control values, open circles; trampled plots, filled circles; research trails, filled squares. Trampling period shown by gray bar. Soil temperature shown by crosses.

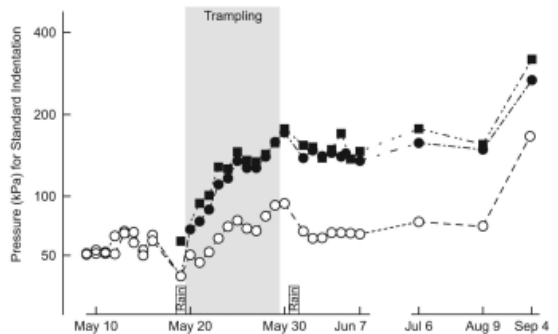


FIGURE 3. Soil compaction assessment over 4 mo in 3 sites (A, H, S – pooled) before, during, and monthly thereafter following daily 5-min standing visits for 10 days in May 2008 (gray vertical band). Heavy rain days are indicated - Rain. Control values, open circles; trampled plots, filled circles; research trails, filled squares.

Soil nematode assemblages, although different in composition at each site, were similarly impacted by trampling. The experimental trampling and trail samples showed a significantly lower proportion of fungivorous to total nematodes than control plots, with 8%, 10%, and 26%, respectively [Wilcoxon Rank Test: $t=3.23$ ($P<0.001$)].

Distribution and association of E. helleborine with large trees—We found 159 *E. helleborine* plants within 3 m of the marked trees of which 44 were flowering stems. Twenty-four plants were found outside the 3 m circles but within the control region: 12 of these plants were flowering. A further 4 plants were observed just outside the 3 m circles and the control region, one of which

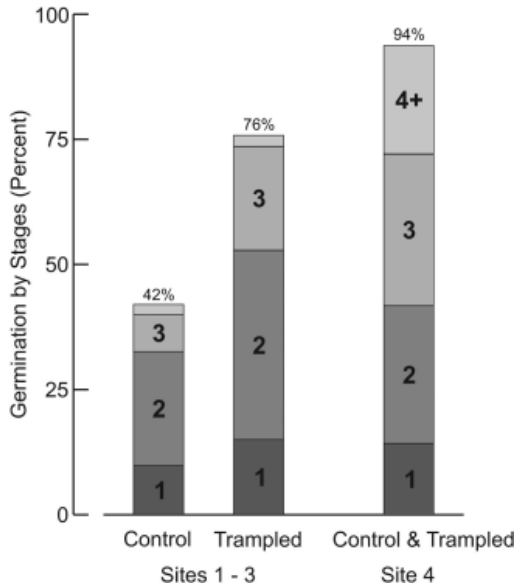


FIGURE 4. Proportional germination and seedling development by stages in packets of *E. helleborine* seeds buried at 5 cm depth for 2 years, with and without 30 footsteps immediately after burial.

was flowering (Fig. 1). Two groups of 5 and 7 plants, respectively, were found within 5 m of *Carya cordiformis* C2 and within 1 m of the fallen top of that tree.

High orchid density was found to be strongly associated with some of the large trees, although there was significant heterogeneity ($X_{102} = 90$, $P \ll 0.001$) in the distribution. The greatest density (52 plants in 60 m²) was observed in the tree cluster consisting of *Acer saccharum* A2 and the two *Tilia americana* L., the four next densest being locations around *Acer* A3, *Carya cordiformis* C1 and the two pairs of close neighbors, *Acer* A7 with the *Quercus rubra* and *Carya* C4 with the *Ostrya virginiana*. In the latter two cases, the orchids were concentrated where the 3 m assessment areas overlapped, with 26 plants in the 15 m² overlapping regions and 24 in the remaining outer 98 m² ($X_2 = 65$, $P \ll 0.001$), and with 9 of the 17 remaining plants associated with the *Quercus* being within 4 m of the trunk of the neighboring *Acer* (Fig. 1).

Impact of trampling on germination of E. helleborine— Three of four seed packet groupings showed a significant positive effect of trampling on subsequent germination (Fig. 4). With these groupings, germination and seedling development after two years of burial were

greater with trampled packets than with controls. While control and trampled plots had been similarly loosened when packets of seeds were buried, only the trampled plots where the soil was still significantly more compacted after two years showed enhanced germination and development. In the fourth packet grouping, located within 2 m of the other groups and close to the felled top of the dead *Carya cordiformis*, being within 4 m of the trunk of that tree, control and trampled seed packets showed equally high (94%) germination and enhanced seedling development. We could not separate the results of control and trampled packet behavior in this group.

Discussion

The use of common plants and animals as models toward the development of ecological and evolutionary theory is not surprising. Many of these model organisms were likely first selected because they were small, common, and easily cultured and propagated (Somerville and Koormeet, 2002). The nematode, *Caenorhabditis elegans* Maupas, the mustard, *Arabidopsis thaliana* (L.) Heynh. and the garden snapdragon, *Antirrhinum majus* L. have become pivotal to our understanding of how many organisms behave at a genetic and genomic level. Orchids can be used to understand flower development (Johansen and Fredericksen, 2002) while mimetic orchids, which employ deception to attract potential pollinators, have proved to be useful models to investigate the molecular mechanisms of floral mimicry (Schlueter & Schiestl, 2008).

Conservation of species presents many challenges, and conservation of biodiversity even more. Conservation is typically managed on a by-species basis with an emphasis on vulnerable species or species at risk yet often without an understanding of how similar yet common species behave in response to shared challenges including disturbance and competition. “Commonness is itself rare,” according to Gaston and Fuller (2007). There may be more of a single species and that species may be widespread, but there are not that many species that are so common or so widespread even within Orchidaceae. Commonness brings with it a risk of neglect or disinterest (Dixon & Backhouse, 2007).

One approach to biodiversity conservation is the creation of protected areas where human activities

can be controlled. Control of visitor movement is not always possible, especially in urban parks or where flowering orchids have become a tourist attraction. In the Mingan Archipelago National Park Reserve of Canada, scientists have been monitoring a population of *Cypripedium passerinum* Richardson. Their observations have revealed that eight of the 12 colonies were below the minimum plant numbers considered necessary to their survival (Nantel & Cantin, 1998). Because the impact of monitoring on the fragile habitat was unknown, the investigators recommended monitoring these eight colonies every three years, whereas a five-year interval was considered appropriate to monitor the other four colonies. Perhaps the knowledge of trampling impact on the habitat will assist park managers with a similar challenge. Until we have an understanding of the impact of natural and anthropogenic disturbance on the establishment and survival of orchids, we cannot adequately protect them nor can we begin to address the challenge of ecosystem conservation.

Long-term study of common temperate terrestrial orchids in Gatineau Park, Québec, Canada, has been used to examine how weather affects pollinator activity and germinable seed production of *Cypripedium parviflorum* var. *pubescens* (Light & MacConaill, 2002, 2006b) and also how visitor traffic, including monitoring activities of the shallow-rooted orchid, would impact the terrestrial ecosystem which could ultimately affect the orchids (Light & MacConaill, 2007, 2008). It is helpful that both *E. helleborine* and *C. parviflorum* var. *pubescens* can and do grow in close proximity in Gatineau Park where weather and natural disturbances such as the catastrophic ice storm of January 1998 were shared phenomena yet elicited different responses by these orchids (Light & MacConaill, 2006b). Where a single *C. parviflorum* var. *pubescens* growing within the *E. helleborine* population of Site 1 was positively affected by the ice storm after a massive litter fall increased the quantity of decaying wood (the plant quadrupled in size over the next 3 years), mature plants of *E. helleborine* were not similarly affected (Light & MacConaill, 2002). Indeed, the earlier natural death of a large tree in the same part of Site 1 and subsequent drought was suggested as a reason for the decline of plant numbers near that tree, whereas the presence of another healthy

large tree about 10 m away was suggested as the reason why some *E. helleborine* were able to survive within 3 m of that tree to re-emerge after 17 years (Light & MacConaill, 2006a,b). The association of emergent *E. helleborine* in 2006 with certain species of large trees is therefore not surprising and underscores the importance of such large trees to this common orchid. The exceptional concentration of orchids between tree pairs and with the 3-tree grouping warrants further investigation.

Despite the seeming weediness of this introduced orchid in North America, seeds do not germinate and grow everywhere they are placed. Our previous experience with experimental *in situ* seed germination (unpublished data) guided the placement of seed packets in the present study. Others working with rare or uncommon species of *Epipactis* Zinn, *Cephalanthera* Rich., and other orchids known to form mycorrhizal associations with trees might find our observations useful.

Our experiment on the impact of trampling on buried seeds of *E. helleborine* has revealed that trampling affected germination and initial seedling development patterns at the 5 cm depth. While control and trampled plots were similarly loosened when packets of seeds were buried, only the trampled plots showed enhanced germination and development. In one specific group of seed packets, located close to a fallen treetop and within 4 m of the trunk of that dead tree, control and trampled seed packets showed equally high germination and enhanced seedling development, which indicates that other factors such as a local mycorrhizal distribution may be implicated (Bidartondo & Read, 2008). Voss (1972) suggested that *E. helleborine* responds positively to disturbance. Wittig and Wittig (2008) wondered why *E. helleborine*, compared to so many other terrestrial species, has been able to colonize human habitats successfully in Central Europe. Perhaps soil compaction alters habitat to its favor as it has similarly colonized ruderal habitats across North America since it was introduced in the 19th century. Anecdotal evidence that this weedy orchid prefers disturbed ground beside trails may not be ill founded.

Our trampling studies have revealed that the fungivorous nematode population decreases with trampling in a variety of soils and forested habitats and

especially so in research trails where this effect has been noted for two years. Disturbance which loosened soil (burying seed packets or the insertion of probes as in our 2006 study) did not have the same impact on the nematode community or on the germination of *E. helleborine* as did disturbance by compaction. The data indicate that trampling somehow disrupts the nematode- fungus interaction in the compacted soil and that this disturbance may be reflected in the fungal community, in particular the ectomycorrhizal community, where orchid seed germination outcomes are determined. This is especially interesting in the case of *E. helleborine* which is known to be mycorrhizal with ectomycorrhizae associated with trees (Selosse *et al.*, 2004). Trampling could somehow be modifying the fungal flora, possibly leading to the predominance of some fungi over others (Brussaard *et al.*, 2001; Bidartondo & Read, 2008). Certain fungi in trampled soil could become less palatable to nematodes or less accessible as a food source, which could happen if the fungal cell walls became thickened and less penetrable by nematode feeding stylets. Additionally, nematodes might not reproduce in trampled areas or they could migrate elsewhere if food choices became limited. It is also possible that the soil bacterial community is changed because of trampling and this impacts differentially upon the fungal assemblage or the nematode community, but this has not been investigated. We did observe a spike in opportunistic bacterivorous nematode numbers after trampling, so this avenue of investigation cannot be discounted (Light & MacConaill, 2007).

There is a growing body of concern expressed by plant and animal conservationists that there may be an overemphasis on single species and rare species conservation with a paucity of understanding of ecosystem components and functioning for even common species (Jiguet & Julliard, 2006; Whiteley *et al.*, 2006; Gaston & Fuller, 2007). There is a real need for understanding of both rare and common species and their role within their respective communities. Protection of a few areas may not be sufficient to support what are now common species. Gaston and Fuller (2007) have argued that we need to pay more attention to common species. This good advice should be heeded by those of us tasked with orchid conservation.

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BOTANIC GARDENS, EDUCATION, AND ORCHID CONSERVATION STRATEGIES: THE NEED FOR A COORDINATED APPROACH

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ABSTRACT. The call for orchid conservation has been heard worldwide by scientists, horticulture professionals, commercial propagators, and passionate private enthusiasts, all with different opinions and approaches on how to tackle this worldwide problem. The news has been dire with the prospects of global warming, habitat destruction, displacement of natives by aggressive exotics, unsustainable plant predation/harvesting, and impotent efforts to enforce legal protections. Considerable thought and effort have gone into specific strategies for orchid conservation, including habitat protection, buying and preserving ‘hot spots’, and the trends toward creating horticultural reserves, both native (Lankester Gardens, Ecominga Foundation, Ecuagenera reserves) and *ex situ* (orchid gardens in Hawaii, collections under glass, etc.) Tactics such as seed-banking, DNA technology, *ex situ* propagation efforts, and eventual reintroductions are inherently dependent on the resources and holdings of botanic gardens and responsible commercial growers. It is such horticultural work that has saved genera such as *Franklinia*, *Torreya*, and *Wollemia* as well as orchid species such as *Paphiopedilum vietnamense*, *Epidendrum ilense*, and *Angraecum longicalcar* from complete extinction. Botanic gardens, while united by certain organizations such as the American Public Garden Association (APGA) and Botanic Garden Conservation International (BGCI), need greater cooperation and coordination of their plant conservation efforts. In addition, it is critical for botanic gardens to engage the scientific community (and vice versa), especially those who can best assess which species are the most endangered. If collections are to be used optimally, the scientific and horticultural communities must become more aware of each other’s assets and priorities. At the first International Orchid Conservation Congress (IOCC), four resolutions were adopted from the Global Strategy for Plant Conservation (GSPC): 1) 90% of all threatened orchids should be secure in *ex situ* collections; 2) 50% should be in active recovery programs; 3) no orchids should be threatened by unsustainable harvesting; and 4) every child should be aware of plant diversity (including orchids) by 2010. How do we assess whether progress has been made toward these admirable goals without a unifying entity to compile the progress, information, and successes? With so much effort going on in so many separate places, such an entity is sorely needed. In addition, the plant conservation community needs more coordination with the efforts of the animal conservation community. It is the goal of many botanic gardens and a specific goal of the Smithsonian Institution to promote the diffusion of knowledge and understanding. To this end, goal # 4 above is a particular priority. While reaching the goal of 100% awareness of plant diversity by 2010 is not possible, we have undertaken various educational initiatives aimed at promoting conservation awareness in our visitors, especially young people. For educators, it is imperative to instill these values at an early age.

RESUMEN. Se ha escuchado a nivel mundial el llamado para la conservación de orquídeas, por parte de científicos, profesionales de horticultura, propagadores comerciales y los apasionados entusiastas privados, todos con diferentes opiniones sobre la manera para atacar este problema global. Las noticias han sido desalentadoras en relación al calentamiento global, destrucción de hábitat, desplazamiento de poblaciones nativas por parte de agresivas, exóticas e insostenibles depredaciones /cosechas de plantas y los esfuerzos impotentes para hacer cumplir las protecciones legales. Se han dedicado muchas ideas y esfuerzos para determinar estrategias específicas para la conservación de orquídeas, incluyendo la protección al hábitat, la compra y preservación de los “puntos calientes,” así como las tendencias hacia la creación de reservas hortícolas, tanto nativas (reservas de los Jardines de Lankester, Fundación EcoMinga, Ecuagenera) y *ex situ* (jardines de orquídeas en Hawái,

colecciones bajo vidrio, etc.). Tácticas como un banco de semillas, tecnología ADN, esfuerzos de propagación *ex situ*, así como eventuales reintroducciones, inherentemente dependen de recursos y propiedades de jardines botánicos y cultivadores comerciales responsables. Es este tipo de trabajo de horticultura el que ha salvado a otros géneros que no corresponden a las orquídeas, tales como *Franklinia*, *Torreya*, *Wollemia*, y especies de orquídeas como *Paphiopedilum vietnamense*, *Epidendrum ilense*, y *Angraecum longicalcar* de la extinción. Los jardines botánicos unidos bajo ciertas organizaciones como la Asociación Americana de Jardines Públicos (American Public Garden Association - APGA) y Jardines Botánicos para la Conservación Internacional (Botanic Garden Conservation International - BGCI), necesitan de una mayor cooperación y coordinación para el éxito de sus esfuerzos para la conservación de plantas. Además, es un aspecto crítico que los jardines botánicos se conecten con la comunidad científica (y viceversa), especialmente aquellos que mejor pueden asesorar sobre aquellas especies más amenazadas. Si las colecciones van a ser utilizadas en forma óptima, las sociedades científicas y de horticultura deben estar más conscientes de sus respectivos activos y prioridades. En el Primer Congreso Internacional para la Conservación de Orquídeas (International Orchid Conservation Congress - IOCC), se adoptaron cuatro resoluciones de la Estrategia Global para la Conservación de Plantas (Global Strategy for Plant Conservation – GSPC en inglés): 1) el 90% de todas las orquídeas amenazadas deberían ser asegurada en colecciones *ex situ*; 2) el 50% debería constar en programas para su recuperación activa; 3) Se debe considerar que las orquídeas están amenazadas cuando su cosecha no es sostenible; y 4) Para el año 2010, cada niño y niña debe estar consciente de lo que es la diversidad de plantas (incluyendo a las orquídeas). ¿C.mo podemos evaluar si se está logrando el progreso hacia estos admirables objetivos sin que exista una entidad que unifique y que pueda recopilar el progreso, la información y el grado de éxitos alcanzado? Al ver el esfuerzo desplegado en tantos lugares separados, es indudable que se necesita urgentemente este tipo de entidad. Constituye la meta de muchos jardines botánicos y una meta específica del Instituto Smithsonian, el promover la diseminación de conocimientos y comprensión. Con este fin en mente, el anterior objetivo #4 constituye una particular prioridad. A pesar de que alcanzar la meta del 100% de conciencia compartida en relación a la diversidad de plantas para el año 2010, hemos iniciado un proyecto que nos permita comprender y finalmente corregir el llamado síndrome de “Ceguera relativa a las Plantas,” bajo el cual la persona promedio no considera que las plantas son seres vivientes (en el mismo sentido de los animales). Mediante el uso de encuestas y entrevistas personales, se está analizando este síndrome generalizado y penetrante para determinar sus características demográficas, hombre/mujer, urbano/rural, jóvenes/ viejos, etc. Es imperativo para los educadores que identifiquen la edad en la cual dichas actitudes empiezan a introducirse en la psiquis de los jóvenes. Es necesario aplicar ciertas técnicas que permitan elevar el grado de conciencia de los individuos de todas las edades en relación a la importancia de la diversidad de las plantas y su conservación. Esto puede tomar la forma de presentaciones, exposición a especies representativas, paseos de campo y otras oportunidades educativas, que permitan cultivar un enfoque mental que comprenda y a la final proteja el mundo natural.

KEY WORDS: orchids, conservation, *ex situ* collections, recovery, education

Worldwide efforts of the wildlife conservation movement have increased dramatically in the last decade. Ambitious projects such as the ‘barcoding of life’ initiative have brought a wide variety of scientists from disparate disciplines together to work on strategies and solutions to tackle some of the world’s most vexing problems (C. Valladares-Padua, unpublished). With the fire of global warming lit beneath us, it is increasingly clear that the present generation of scientists, conservationists, and legislators have the obligation to engage in innovative thinking to preserve

species and habitat diversity as well as to do everything in their power to change course before mass extinction occurs (W. F. Laurance, unpublished). In the animal conservation community, many protocols have been adapted that could help the botanical community achieve its conservation goals. The purpose here is to create awareness of some of the successes and failures of the zoological community in the hope that we can learn from and adapt these practices to improve our own efforts to protect imperiled plants.

The orchid community, by nature of the passion

evoked by this charismatic plant family, has been a leader in plant conservation efforts. With a colorful history of rampant wild collection, habitat destruction, pollinator specificity, and displaying unparalleled species and population diversity, an awareness of the need for conservation policy has been long understood among orchidophiles. Organizations such as the IUCN Orchid Specialist Group, Orchid Conservation International (<http://www.orchidconservation.org/OSG/>), the Orchid Conservation Alliance, and the Ecominga Foundation (<http://ecominga.net/>) have supported numerous projects around the world aimed at preserving orchid habitats, particularly IPAs (Important Plant Areas), that are natural hotspots for orchid species diversity. Many other organizations and individuals are working in virtual isolation toward the same goals. As admirable as these efforts are, more coordination, collaboration, and focus are necessary to achieve desired long-term goals.

In addition to preserving IPAs, orchid conservation efforts are necessarily dependent on the success of reforestation and habitat reconstruction projects. If the ultimate goal of our conservation efforts is to return extirpated orchids to their original wild habitats, then such efforts are futile when those habitats no longer exist. Without a suitable ecosystem to support it, including the entire milieu of plants, insects, and other pollinators, orchid plants may survive for a time, but they will not thrive and be able to reproduce. The work of Chazdon (2009) showed that rebuilding an ecosystem may not be as difficult as previously thought. Her work focused on secondary forests in Costa Rica. Though not as ideal for habitat as primary forest, such forests can still contain up to 80% of the tree species found in primary forest. If left alone or supplemented with absent species, these forests could still be excellent habitats for reintroduction of orchids. At the very least, such forests can serve as a buffer between primary forests and agricultural monocultures.

Botanical gardens have a crucial role to play in orchid conservation strategy. The usual role of botanic garden collections is for horticultural display, visitor enjoyment, and education. This contribution should not be underestimated as it is likely the only opportunity for the majority of people to witness the diversity of plants outside their own heavily altered environment. Many present-day leaders in the botanical community got their

first glimpse of the plants in which they now specialize while visiting a botanic garden. The development of kid-friendly exhibits such as the USBG's Alphabet Garden of Orchids as well as the Smithsonian Institution's development of orchid-related texts in grade-school and highschool textbooks should pay off in greater public consciousness and the development of the next generation of conservation biologists.

Such garden collections, if properly documented and kept disease-free, are also repositories for endangered species and as such can serve as a source for genetic material for *ex situ* breeding programs. Often considerably less well funded than zoos, with a few exceptions, most botanical gardens have not yet realized their conservation potential in the same way as their animal counterparts. Organizations such as Botanic Gardens Conservation International (<http://www.bgci.org>) and the American Public Garden Association (APGA; <http://www.publicgardens.org/>) seek to advance plant conservation efforts by following and promoting the advancement of the Global Strategy for Plant Conservation (<http://www.cbd.int/gspc/>). While they've been successful in achieving these goals in developed countries, most of the orchid diversity occurs in less-developed nations where the economic climate precludes prioritizing plant conservation. Small details such as the need for translations into English are often keeping thousands of species from being red-listed, the first important step to their protection.

More specifically, at the first International Orchid Conservation Congress (IOCC), four resolutions were adopted from the Global Strategy for Plant Conservation (GSPC):

1. 90% of all threatened orchid species should be secure in *ex situ* collections. While progress has definitely been made toward achieving this admirable goal, it seems certain that with so many endemic species in countries such as Ecuador that have yet to be even red-listed, we haven't as yet placed close to 90% of threatened orchid species in *ex situ* situations. It is incumbent on institutions in the developed world to help get these species listed and sent to appropriate botanic gardens to ensure their survival.
2. 50% of threatened orchids should be in active recovery programs. Since this goal depends on

resolution #1 having been achieved, we find ourselves unable to progress well with this important task. Plant reintroductions and recovery programs are considerably more complicated and fraught with difficulty than we thought 10 years ago. The need to restore forest (and other) habitats as well as the species diversity within them requires the participation of a large number of biologists to understand fully the implications of such projects on the ecology of any given region. However, a few such projects have been successful, including efforts by Henry Doorly Zoo ([http://www.omahazoo.com/index.php?p=c](http://www.omahazoo.com/index.php?p=conservation&s=rareplantresearch)onservation&s=rareplantresearch) and the Royal Botanic Gardens, Kew, to recover and reintroduce orchids in Madagascar, the Ecominga foundation in Ecuador, and similar initiatives in Costa Rica and Panama.

3. No orchids should be threatened by unsustainable harvesting. While much of the illegal harvesting of wild orchids has been curtailed by the enforcement of laws resulting from the Convention on the International Trade in Endangered Species (CITES), some illicit and unsustainable activity persists in the orchid world. The most recent evidence of this is the voracious pillaging of the habitats of *Paphiopedilum vietnamense* (O.Gruss & Perner) and *Phragmipedium kovachii* J.T.Atwood, Dalstr.m & Ric.Fern.ndez. These horticulturally important plants were plundered mercilessly by commercial orchid companies and private individuals. Efforts to control their trade were undermined in several ways. In the case of *P. vietnamense*, the local authorities were unable to control or enforce laws against wild collection, resulting in their virtual extirpation in the wild. In addition, smuggled plants acquired by CITES rescue centers were propagated in the hope that inexpensive, artificially produced propagules would reduce wild-collection pressure. Although this effort was successful, it also created a smokescreen for many illegally collected plants to enter the trade. Conservation efforts for *Phragmipedium kovachii* have been more successful due to the active participation of the Peruvian government in the protection and propagation effort for this

valuable species. The lesson learned here is that the engagement of local stakeholders will usually result in better stewardship of endangered species both in the wild and in cultivation.

4. Every child should be aware of plant diversity (including orchids) by 2010. Although reaching every child in the world is a daunting goal, admirable progress has been made on this resolution. With the worldwide consciousness raised in the need for conservation of species, remediation of global warming, and the value of our natural systems, it seems likely that the next generation of students will be keenly aware of the importance of biological (including botanical) diversity for the continued health of our planet.

In contrast to their zoological counterpart, the Association of Zoos and Aquariums (AZA), botanical organizations do not require their member gardens to engage in conservation efforts with their collections, although initiatives of APGA such as the NAPCC (North American Plant Collections Consortium) promise a future database where germplasm from a network of collections can eventually be accessed and used for breeding purposes. Accreditation by the AZA is contingent on the participation of all zoos possessing endangered species in their collection in captive breeding programs. Zoo staff from all participating institutions are required to follow specific protocol and have input in international SSPs (Species Survival Plans), TAGs (Taxon Advisory Groups), and SAGs (Scientific Advisory Groups) for their endangered holdings. These are associated with databases that record husbandry and collection data to prevent inbreeding and encourage genetic diversity within captive populations. The plant community could benefit greatly from adopting similar tactics for the plant resources in botanic gardens and other orchid species collections.

Much work has been done in the U.K. and U.S. with the cryopreservation and banking of orchid seed. Projects such as the OSSSU (Orchid Seed Stores for Sustainable Use) and the Orchid Seedbank Project may indeed be the best hope for preserving plant populations. Individual plants in botanic gardens have their worth but represent only one set of genes. Space requirements would certainly limit the quantity

of any given taxon in a botanic garden. Seedbanking also preserves some genetic diversity and should be encouraged and supported for eventual reintroduction projects.

Other lessons to be learned from the animal world include an analysis of their prowess at outreach, fund-raising, and public engagement. The Internet represents a vast, untapped opportunity to harness a tremendous interest in orchids that clearly exists when all the myriad websites devoted to orchid species culture and discussion are considered. The website for the organization Amphibian Ark (<http://www.amphibianark.org>) is a prime example of how to use the Internet as a fund-raising tool, building the awareness of the need for public engagement and reaching out to many constituencies, especially children. Another virtually untapped source of outreach is the media. More articles need to be written in popular magazines rather than scientific journals if conservation goals are to be understood and promulgated among more people. And, finally, wild animals have been featured on television for generations and have notable personalities and celebrities advocating their conservation, yet the plant world has only 'gardening-type' programming. With the astounding array of ecological niches, deceptive

pollination strategies, and charismatic young people deeply involved in orchid conservation biology, we should be producing and promoting programming that will engage the public, raise their consciousness, and directly involve them in orchid conservation efforts.

Perhaps most importantly, it has become apparent within the animal conservation community that a more holistic approach to conservation is necessary for success. The case history of the Takhi (the Mongolian wild horse) is a prime example of a reintroduction project that focused specifically on the animal without fully taking into consideration its foraging needs (<http://nationalzoo.si.edu/ConservationAndScience/CRC/>). With just enough vegetation to support the dominant male and his females, the rest of the reintroduced herd starved. The inclusion of botanical expertise to restore the habitat of the Takhi prior to reintroduction would have produced better results. It will always be necessary for animal and plant biologists to collaborate on conservation projects in the same way that plants and animals, on the most basic level, need each other to survive. It's imperative that botanists and zoologists develop plans (and apply for grants) together that address the interconnected needs of flora and fauna.

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<http://www.omahazoo.com/index.php?p=conservation&s=rareplantresearch> np accessed 10/2008

PRELIMINARY MOLECULAR PHYLOGENETICS OF *SOBRALIA* AND RELATIVES (ORCHIDACEAE: SOBRALIEAE)

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ABSTRACT. With over 200 species, the orchid tribe Sobralieae is a major constituent of the Neotropical flora. As currently circumscribed, the tribe includes four genera: *Elleanthus*, *Epilyna*, *Sertifera*, and *Sobralia*. Most species of these four genera typically produce long, cane-like stems but differ drastically in flower size and inflorescence structure. DNA sequence data support the monophyly of *Elleanthus*, *Epilyna*, and *Sertifera* but not *Sobralia*, which is a polyphyletic assemblage traditionally placed together due to relatively large flower size. Details of inflorescence structure provide characters that can easily distinguish the different clades of *Sobralia*. The misleading characteristic of flower size is probably due to at least several shifts in pollination syndrome within the tribe. With few exceptions, species of *Sobralia* predominantly offer no reward and are pollinated by bees. *Elleanthus* and *Sertifera* are small-flowered and mostly pollinated by hummingbirds with legitimate rewards. Nothing is known of pollination in *Epilyna*. Understanding the evolution of shifts in pollination syndrome will require more empirical observations of pollination within Sobralieae. In addition, increased taxon sampling and improved phylogenetic resolution are needed before generic realignments are made.

RESUMEN. Con más de 200 especies, la tribu de orquídeas Sobralieae es un componente importante de la riqueza florística de los neotrópicos. Actualmente esta tribu está constituida por cuatro géneros: *Elleanthus*, *Epilyna*, *Sertifera*, y *Sobralia*. Las plantas de éstos cuatro géneros generalmente producen tallos largos como cañas, pero difieren en forma drástica en el tamaño de la flor y la estructura de las inflorescencias. Datos de ADN apoyan la monofilia de *Elleanthus*, *Epilyna*, y *Sertifera*, pero no de *Sobralia*. *Sobralia* es un ensamblaje polifilético, tradicionalmente circunscrito por el gran tamaño de sus flores. Los detalles de la morfología floral y la posición de la inflorescencia proporcionan caracteres que fácilmente permiten distinguir los diferentes clados de *Sobralia*. El tamaño de la flor y ciertas otras características superficiales probablemente han sufrido cambios evolutivos en respuesta a cambios en el síndrome de polinización dentro de la tribu. La mayoría de las especies de *Sobralia* no ofrecen ninguna recompensa y son polinizadas por abejas en busca de néctar. *Elleanthus* y *Sertifera* tienen flores pequeñas que aparentemente son polinizadas por colibríes, en estos dos géneros las flores ofrecen néctar. No se conoce nada sobre la polinización de *Epilyna*. Mas observaciones empíricas de los polinizadores de Sobralieae son necesarias para entender la evolución de los síndromes de polinización, y requerirá un mayor muestreo de especies y una mejor resolución filogenética antes de realizar recircunscripciones genéricas.

KEY WORDS: Orchidaceae, Sobralieae, *Sobralia*, phylogenetics

Tribe Sobralieae, described by Pfitzer in 1887, has been recognized as a natural group, at least in part. For part of its nomenclatural history it has been known as subtribe Sobraliinae (although placed in several different tribes). Dressler (1981) placed his subtribe Sobraliinae in tribe Arethuseae based on

symplesiomorphies such as presence of corms, plicate leaves, and eight soft pollinia (although he also included aberrant genera such as *Arpophyllum* and *Xerorchis*). Dressler (1993) later placed subtribe Sobraliinae in tribe Epidendreae based on the distinctive velamen and seed morphology. In general, variation in taxonomic placement of Sobralieae has been associated with other basal members of subfamily Epidendroideae based on plesiomorphic subfamilial characters. More recent and objective phylogenetic analyses using DNA data have demonstrated that Sobralieae are basal members of the subfamily Epidendroideae, closely related to genera such as *Tropidia* (Cameron *et al.*, 1999; Cameron, 2002, 2004). Because this group is not closely related to other taxa in tribes Epidendreae and Arethuseae, the former subtribe Sobraliinae is now recognized as a tribe (see Pridgeon *et al.*, 2005).

Tribe Sobralieae consists of only four genera of unequal species richness. Two genera, *Elleanthus* C.Presl. and *Sobralia* Ruiz & Pav., each consist of about 100 species, whereas the other two genera, *Epilyna* Schltr. and *Sertifera* Lindl. & Rchb.f., each consist of less than 10 species. The tribe as a whole is widely distributed in tropical America. *Sertifera* is restricted to relatively high elevations in the northern Andes. *Epilyna* is found in southern Central America and northern South America. *Elleanthus* is distributed throughout tropical America, and *Sobralia* is similar in distribution except for notable absence in the West Indies.

Although some vegetative traits are useful for identifying species or groups within Sobralieae, there is ample homoplasy in vegetative morphology among distantly related taxa. Genera have been delimited on the basis of relatively few gross floral characters (Fig. 1). *Sobralia* has largely been recognized based on relatively large flowers. The other three genera (*Elleanthus*, *Epilyna*, *Sertifera*) all have relatively small flowers. This criterion is misleading and has been shown to result in the circumscription of polyphyletic groups based on homoplasious character evolution (e.g., Johnson *et al.*, 1998). Because there has been such a poor understanding of generic circumscription in Sobralieae and no robustly taxon-sampled phylogenetic analysis of the tribe, we addressed phylogenetic relationships within the tribe. We hypothesized that floral size would not be adequate

for reciprocal monophyly in these genera because the polarity of such a character would make one state symplesiomorphic. Therefore, the purpose of this study was to provide a phylogenetic framework in which to understand the evolution of morphological variation in tribe Sobralieae.

Materials and methods

Taxon sampling — Specimens were obtained from wild-collected and cultivated plants (Table 1). Sampling of *Elleanthus*, *Epilyna*, *Sertifera*, and *Sobralia* included 42 species. Outgroups included three other genera of basal Epidendroid tribes — Neottieae (*Palmorchis*), Arethuseae (*Bletilla*), and Tropidieae (*Tropidia*). Outgroups were chosen based on phylogenetic placement of *Sobralia* and *Elleanthus* in previous work (Cameron *et al.*, 1999; Cameron, 2002; Chase *et al.*, 2003; Cameron, 2004).

Extractions, amplification and sequencing — All freshly collected material was preserved in silica gel (Chase & Hills, 1991). Genomic DNA was extracted using a modified cetyl trimethylammonium bromide (CTAB) technique (Doyle & Doyle, 1987), scaled to a 1 mL volume reaction. Approximately 10 mg of dried tissue were ground in 1 mL of CTAB 2X buffer and either 8 μ L of β -mercaptoethanol or 10 μ L of proteinase-K. Some total DNAs were then cleaned with Qiagen QIAquick PCR purification columns to remove any inhibitory secondary compounds. Amplifications were performed using a Biometra Tgradient or an Eppendorf Mastercycler EP Gradient S thermocycler and Sigma brand reagents in 25 μ L volumes with the following reaction components for ITS: 0.5-1.0 μ L template DNA (~10-100 ng), 11 μ L water, 6.5 μ L 5M Betaine, 2.5 μ L 10X buffer, 3 μ L MgCl₂ (25mM), 0.5 μ L of 10 μ M dNTPs, 0.5 μ L each of 10 μ M primers, and 0.5 units *Taq*. For the plastid regions the following reaction components were used: 0.5-1.0 μ L template DNA (~10-100 ng), 16-17.5 μ L water, 2.5 μ L 10X buffer, 2-3 μ L MgCl₂ (25mM), 0.5 μ L of 10 μ M dNTPs, 0.5 μ L each of 10 μ M primers, and 0.5 units *Taq*.

nrITS (ITS 1 + 5.8S rDNA+ ITS 2) — This region was amplified with a touchdown protocol using the parameters 94 C, 2 min; 15X (94 C, 1 min; 76 C, 1 min, reducing 1 C per cycle; 72 C, 1 min); 21X (94 C, 1 min; 59 C, 1 min; 72 C, 1 min); 72 C, 3 min with the

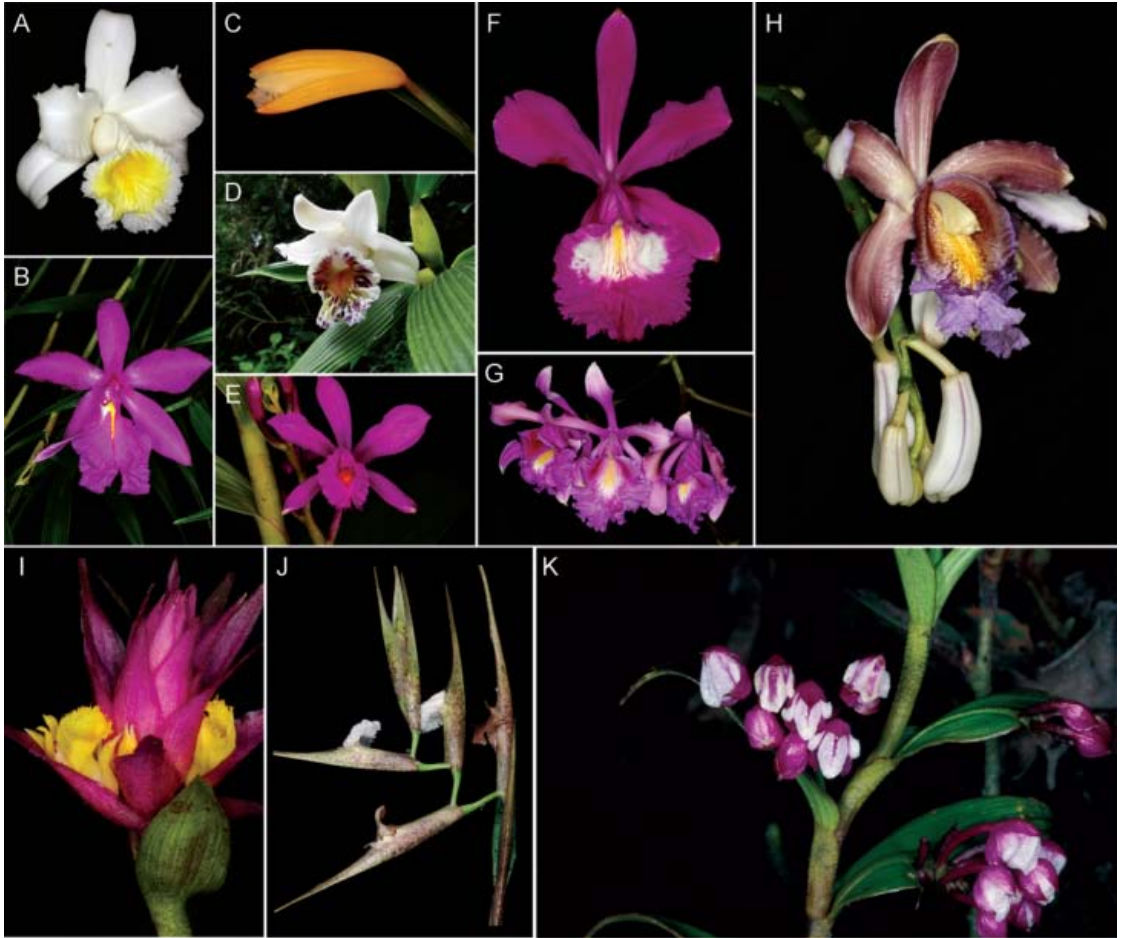


FIGURE 1. Floral diversity of tribe Sobralieae. There is extensive variation in the “core” group of *Sobralia*, such as in A) *S. citrea*, B) *S. callosa*, C) *S. crocea*, and D) *S. luerorum*. Various members of *Sobralia* sect. *Sobralia* include E) *S. ciliata*, F) *S. portillae*, G) *S. mandonii*, and H) *S. caloglossa* (not sampled in this study, but unpublished data place this species in a clade with *S. mandonii* and *S. dichotoma*). Most members of the genus *Elleanthus* have brightly colored bracts and flowers as in I) *E. caravata*, but some species have small white flowers and brownish bracts as in J) *E. lancifolius*. K) Species of the genus *Sertifera* all have flowers that are brightly colored pink and white.

primers 17SE (ACG AAT TCA TGG TCC GGT GAA GTG TTC G) and 26SE (TAG AAT TCC CCG GTT CGC TCG CCG TTA C) from Sun *et al.* (1994).

trnS^{GCU}-trnG^{UCC} – This region was amplified with the parameters 94 C, 3 min; 33X (94 C, 30 sec; 50 C, 30 sec; 72 C, 2 min); 72 C, 3 min, with the primers trnSGCU (AGA TAG GGA TTC GAA CCC TCG GT) and 3'trnG^{UCC} (GTA GCG GGA ATC GAA CCC GCA TC) from Shaw *et al.* (2005).

ycf1 – We sequenced a ca. 1500 base-pair (bp) portion from the 3' end (Neubig *et al.*, 2009). This region was amplified using a “touchdown” protocol with the

parameters 94 C, 3 min; 8X (94 C, 30 sec; 60-51 C, 1 min; 72 C, 3 min); 30X (94 C, 30 sec; 50 C, 1 min; 72 C, 3 min); 72 C, 3 min, with primers 3720F (TAC GTA TGT AAT GAA CGA ATG G) and 5500R (GCT GTT ATT GGC ATC AAA CCA ATA GCG). Additional internal primers intF (GAT CTG GAC CAA TGC ACA TAT T) and intR (TTT GAT TGG GAT GAT CCA AGG) were also required for sequencing.

PCR products were cleaned with Microclean™ (The Gel Company, San Francisco, CA, USA) following the manufacturer's protocols, eluted with 50 μL of 10 mM Tris-HCl (pH 8.5) and stored at 4 C. Purified

TABLE 1. Species names and voucher information, including herbarium of voucher deposition, for material used in this study.

Taxon	Voucher
<i>Bletilla striata</i> (Thunb. ex Murray) Rchb.f.	Neubig 1-2006 (FLAS)
<i>Elleanthus aurantiacus</i> (Lindl.) Rchb.f.	Whitten 1611 (FLAS)
<i>Elleanthus capitatellus</i> Dressler	Neubig 201 (FLAS)
<i>Elleanthus caricoides</i> Nash	Blanco 3106 (FLAS)
<i>Elleanthus caravata</i> (Aubl.) Rchb.f.	Neubig 202 (FLAS)
<i>Elleanthus conifer</i> (Rchb.f. & Warsz.) Rchb.f.	Blanco 2527 (FLAS)
<i>Elleanthus cynarocephalus</i> (Rchb. f.) Rchb.f.	Blanco 3105 (FLAS)
<i>Elleanthus lancifolius</i> C.Presl	Blanco 2918 (FLAS)
<i>Elleanthus oliganthus</i> (Poepp. & Endl.) Rchb.f.	Whitten 2861 (FLAS)
<i>Elleanthus cf. virgatus</i> (Rchb.f.) C. Schweinf.	Whitten 1740 (FLAS)
<i>Elleanthus cf. purpureus</i> (Rchb.f.) Rchb.f.	Whitten 3538 (FLAS)
<i>Elleanthus stolonifer</i> Barringer	Blanco 2934 (FLAS)
<i>Elleanthus tricallosus</i> Ames & C.Schweinf.	Blanco 2961 (FLAS)
<i>Epilyna hirtzii</i> Dodson	Whitten 2938 (FLAS)
<i>Epilyna jimenezii</i> Schltr.	Blanco 2997 (FLAS)
<i>Palmorchis powellii</i> (Ames) C.Schweinf. & Correll	Vargas 2115 (INB)
<i>Sertifera colombiana</i> Schltr.	Whitten 2937 (FLAS)
<i>Sobralia allenii</i> L.O.Williams	Whitten 2834 (FLAS)
<i>Sobralia bouchei</i> Ames & C.Schweinf.	Blanco 3000 (FLAS)
<i>Sobralia callosa</i> L.O.Williams	Blanco 3021 (FLAS)
<i>Sobralia chrysostoma</i> Dressler	Neubig 213 (FLAS)
<i>Sobralia ciliata</i> (C. Presl) C.Schweinf. & Foldats	Whitten 3529 (FLAS)
<i>Sobralia citrea</i> Dressler	Blanco 3030 (FLAS)
<i>Sobralia crispissima</i> Dressler	Whitten 2973 (FLAS)
<i>Sobralia crocea</i> (Poepp. & Endl.) Rchb.f.	Whitten 1578 (FLAS)
<i>Sobralia decora</i> Bateman	Whitten 2862 (FLAS)
<i>Sobralia dichotoma</i> Ruiz & Pav.	Whitten 3532 (FLAS)
<i>Sobralia dorbignyana</i> Rchb.f.	Trujillo 276 (HURP)
<i>Sobralia klotzscheana</i> Rchb.f.	Blanco 3011 (FLAS)
<i>Sobralia labiata</i> Warsz. & Rchb.f.	Whitten 2832 (FLAS)
<i>Sobralia lancea</i> Garay	Whitten 2869 (FLAS)

Taxon	Voucher
<i>Sobralia leucoxantha</i> Rchb.f.	Blanco 2675 (FLAS)
<i>Sobralia liliastrum</i> Lindl.	Koehler 34146 (ESA)
<i>Sobralia luerorum</i> Dodson	Whitten 2729 (FLAS)
<i>Sobralia macrophylla</i> Rchb.f.	Blanco 3022 (FLAS)
<i>Sobralia mandonii</i> Rchb. f.	Whitten 3247 (FLAS)
<i>Sobralia mucronata</i> Ames & C.Schweinf.	Blanco 2971 (FLAS)
<i>Sobralia portillae</i> Christenson	Whitten 2433 (FLAS)
<i>Sobralia quinata</i> Dressler	Pupulin 3644 (USJ-L)
<i>Sobralia recta</i> Dressler	Whitten 2851 (FLAS)
<i>Sobralia rosea</i> Poepp. & Endl.	unvouchered
<i>Sobralia undatocarinata</i> C.Schweinf.	Maduro & Olmos 227(FLAS)
<i>Sobralia warszewiczii</i> Rchb f.	Blanco 2676 (FLAS)
<i>Sobralia yauaperyensis</i> Barb.Rodr.	Blanco 3023 (FLAS)
<i>Tropidia polystachya</i> (Sw.) Ames	Whitten 2830 (FLAS)

PCR products were then cycle-sequenced using the parameters 96 C, 10 sec; 25X (96 C, 10 sec; 50 C, 5 sec; 60 C, 4 min), with mix of 3 μ L water, 1 μ L fluorescent Big Dye dideoxy terminator, 2 μ L Better Buffer™ (The Gel Company), 1 μ L template and 0.5 μ L primer. Cycle sequencing products were cleaned using ExoSAP™ (USB Corporation, OH, USA) following the manufacturer's protocols. Purified cycle sequencing products were directly sequenced on an ABI 377, 3100 or 3130 automated sequencer according to the manufacturer's protocols (Applied Biosystems, Foster City, CA, USA). Electropherograms were edited and assembled using Sequencher 4.9™ (GeneCodes, Ann Arbor, MI, USA). All sequences were deposited in GenBank (Table 1).

Data analysis – Sequence data were manually aligned using Se-Al v2.0a11 (Rambaut, 1996). No sequence data were excluded from analyses. Indels (insertions/deletions) were not coded as characters. Analyses were performed using PAUP*4.0b10 (Swofford, 1999). Fitch parsimony (unordered characters with equal weights; Fitch, 1971) analyses used a heuristic search strategy consisted of branch swapping by

tree bisection reconnection (TBR), Deltran character optimization, stepwise addition with 1000 random-addition replicates holding 5 trees at each step, and saving multiple trees (MulTrees). Levels of support were assessed using the bootstrap (Felsenstein, 1985). Bootstrap percentages under parsimony were estimated with 1000 bootstrap replicates, using TBR swapping for 50 random-addition replicates per bootstrap replicate. For maximum likelihood (ML), Modeltest (Posada & Crandall, 1998) was used to determine the appropriate model for analysis using all combined data under the Akaike Information Criterion. ML analyses were performed using a TrN+I+ Γ model for the ITS data set, a K81uf+I+ Γ model for the combined plastid data set, and TIM+I+ Γ model for the combined three-gene data set. Bootstrap percentages under ML were estimated with 100 bootstrap replicates, using TBR swapping for one random-addition replicate per bootstrap replicate.

All analyses were performed for data sets including ITS only, plastid only, and all data combined. Data congruence was tested using the partition homogeneity test (HTF) in PAUP*4.0b10 (Swofford, 1999) as described by Johnson and Soltis (1998). Heuristic

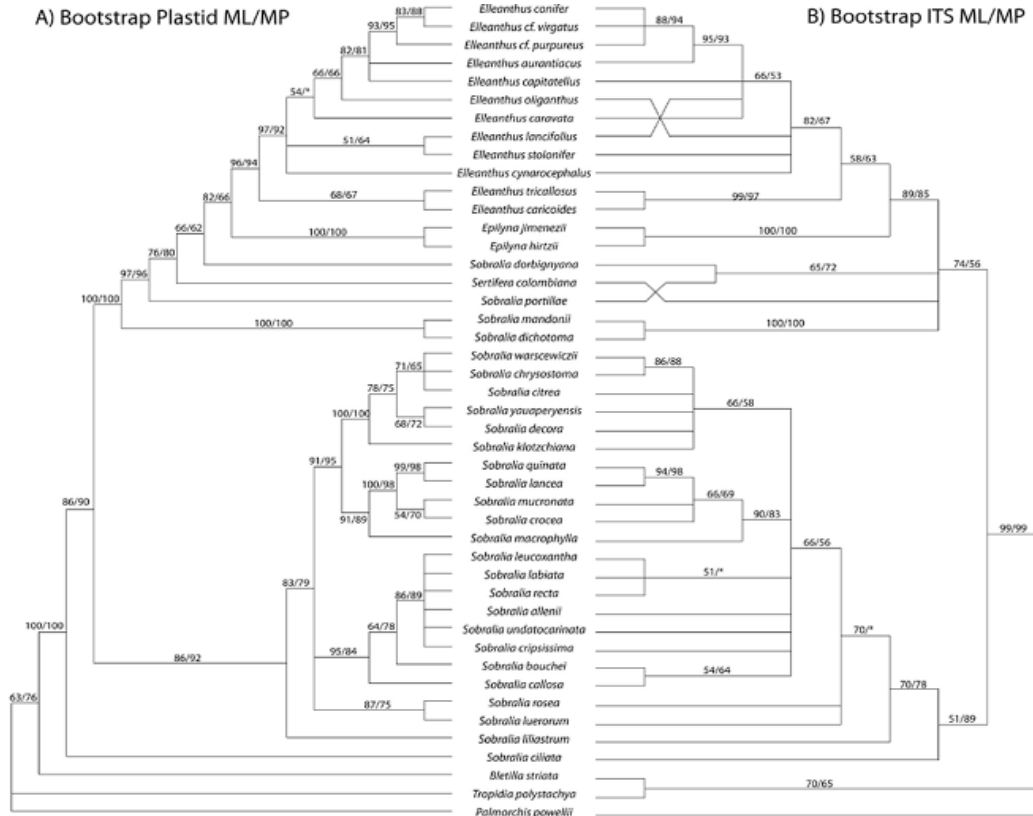


FIGURE 2. Comparative phylogenetic structure among data partitions in Sobralieae. A) From combined plastid data set (*ycf1* and *trnS-G*). B) From nuclear ribosomal internal transcribed spacer (ITS). Numbers above or below branches indicate maximum likelihood and parsimony bootstrap percentages, respectively. An asterisk represents bootstrap support of less than 50%.

searches for the HTF tests were performed using 100 replicates and TBR branch-swapping. Probability values lower than 0.05 were used to identify data sets that were significantly different from one another.

Results

The aligned length of the ITS data set was 892 bp. Of these, 222 were parsimony-informative (24.9%). Fitch parsimony analysis of the ITS region found 100 equally parsimonious trees of 798 steps (consistency index (CI) = 0.589, retention index (RI) = 0.753). The aligned length of the combined plastid data set (*trnS-G* and *ycf1*) data set was 2919 bp. Of these, 250 were parsimony-informative (8.6%). Fitch parsimony analysis of the combined plastid data set found 100 equally parsimonious trees of 1112 steps (CI = 0.772, RI = 0.794). The aligned length of the combined (three

DNA regions) data set (ITS, *trnSG*, and *ycf1*) was 3811 bp. Of these, 472 were potentially parsimony-informative (12.4%). Parsimony analysis of all three DNA regions found 36 equally parsimonious trees of 1926 steps (CI = 0.690, RI = 0.767).

Maximum likelihood analysis of ITS only (not presented), plastid data only (not presented), and all three regions ($-\ln L = 16599.46$) yielded trees similar in topology to parsimony. Bootstrap support for all nodes was similar to that from parsimony. The only exception is in the relative placement of *Sobralia ciliata* in plastid versus ITS data (Fig. 2).

Partition homogeneity tests showed mixed results for congruence among the different partitions of these data. The test comparing ITS and the combined plastid data showed significant incongruence compared with random partitions of the same size ($P=0.03$, $\alpha=0.05$).

However, various combinations of each of the three individual data sets did not indicate significant incongruence (ITS/*trnS-G* $P=0.10$; ITS/*ycf1* $P=0.13$; *ycf1/trnS-G* $P=0.05$). A visual comparison of bootstrap percentages between the different data sets (Fig. 2) indicates that there are only a few examples of strong incongruence. For example, *Sobralia ciliata* is sister to the “core” group of *Sobralia* according to ITS but sister to the rest of the tribe in the plastid data set. Other incongruencies can be found in the relative positions of *S. dorbignyana*, *S. portillae*, *S. mandonii*, *S. dichotoma*, and *Sertifera colombiana*. All data were combined because the partition homogeneity test has been demonstrated to be overly sensitive (Graham *et al.*, 1998; Reeves *et al.*, 2001) and because a total evidence approach yields highly resolved and relatively strongly supported topology.

With limited outgroup taxon sampling, relationships among the basal Epidendroideae tribes Neottieae (*Palmorchis*), Tropidieae (*Tropidia*), Arethuseae (*Bletilla*), and Sobralieae remain unclear. However, tribe Sobralieae is monophyletic in all data sets.

Within Sobralieae, there are many consistent features among different data sets. The “core” group of *Sobralia* (see Fig. 3, 4), *Elleanthus*, and *Epilyna* are all consistently monophyletic. Because only one sample of *Sertifera* was used in this study, monophyly of the genus could not be determined. Inconsistent features of phylogenetic topology are centered on *Sobralia* species within section *Sobralia*: *S. dichotoma*, *S. ciliata*, *S. dorbignyana*, *S. mandonii*, and *S. portillae*. These species have basal positions within the trees; however, their relative position to each other varies among different data sets.

Discussion

Morphological characters supporting the monophyly of Sobralieae include an elongate cane-like stem and flowers with two calli at the base of the lip. Within Sobralieae, *Elleanthus* and *Epilyna* are both monophyletic, but *Sobralia* is polyphyletic. We sought morphological features that might distinguish the various clades that have been taxonomically included in *Sobralia*. These features are discussed below.

Inflorescence structure – Inflorescences in Sobralieae may be axillary or terminal. Terminal inflorescences

are formed at the apex of a shoot and axillary inflorescences are borne from axillary buds, basal to the shoot terminus. The distinction between these two positions can be blurred in some plant groups, but in Sobralieae, the difference is usually clear (see Fig. 1, 4 for variation in inflorescence structure). However, in a few species (e.g., *Sobralia dorbygniana*), both terminal and axillary inflorescences are produced because the inflorescence is a compound panicle. Inflorescences also have bracts (leaf-derived structures), and these can vary in size and shape. Furthermore, the axis of an inflorescence (i.e., the rachis) may be highly condensed (capitate in some species of *Elleanthus*) or elongate, branched or unbranched, erect or (less commonly) nodding, and may have either spiral or distichous phyllotaxy. In a few species of *Elleanthus*, specialized short shoots with reduced leaves bear the (terminal) inflorescences, whereas the taller, leafy shoots do not produce inflorescences at all.

In Sobralieae, all of these inflorescence structural variants exist in some combination. These differences are presented in the simplified illustrations of Figure 4. As delimited in Figure 3, the “core *Sobralia*” is a group distinguished by two main types of inflorescence morphology. Both types are terminal, but in species such as *S. rosea* and *S. luerorum* (*S. sect. Racemosae*) the floral displays are strongly distichous and the rachis is fractiflex (“zigzag”) with relatively large bracts. *Sobralia liliastrum* also has this inflorescence morphology, and when combined with *S. rosea* and *S. luerorum*, this assemblage is paraphyletic. In the remainder of “core *Sobralia*,” the inflorescence rachis is highly condensed, such that the internodes of the rachis are extremely short (often 1–2 mm). The resulting morphology appears acaulescent with relatively large bracts. This condensed inflorescence is present in many *Sobralia* with ephemeral flowers.

In the combined analysis (Fig. 3, 4), *Sobralia ciliata* is sister to “core *Sobralia*,” whereas *S. dichotoma* and *S. mandonii* are sister to the remainder of the tribe. These three species have all been placed in *S. sect. Sobralia*. In addition to the genus *Sertifera*, these species all have axillary inflorescences that may or may not branch to form panicles as well as relatively small inflorescence bracts. Two additional species of

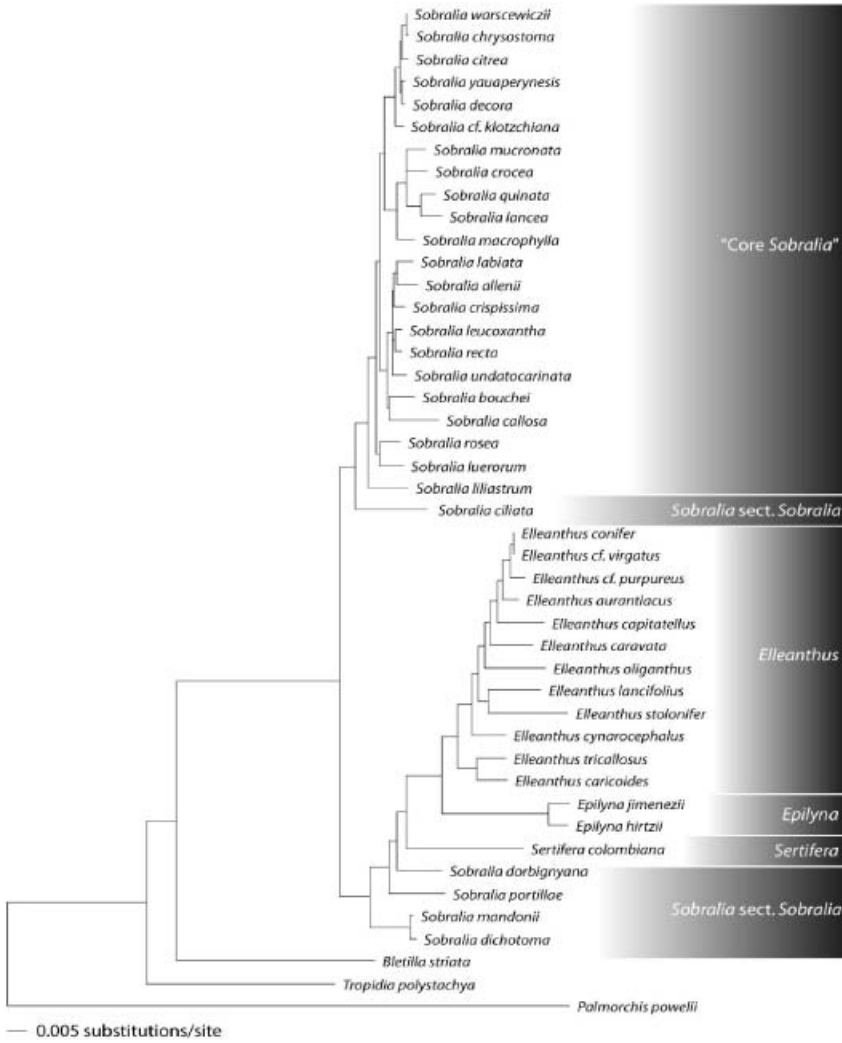


FIGURE 3. The single tree (phylogram) of Sobralieae found in a heuristic maximum likelihood search using all three DNA regions (ITS, *trnS-G*, and *ycf1*).

S. sect. *Sobralia* (*S. dorbignyana* and *S. portillae*) have terminal inflorescences. This feature is shared with virtually all species of *Epilyna* and *Elleanthus*. *Elleanthus* has the most variable inflorescences in the whole tribe. *Elleanthus* inflorescences can be distichous or spirally arranged, capitate to loosely racemose, and can be oriented downwards, upwards or even horizontally (parallel to the ground).

The evolutionary trends in each of the two large clades of Sobralieae demonstrate the plesiomorphic condition of axillary inflorescences. This apparently symplesiomorphic grade across both major clades

is represented by some taxa of *S.* sect. *Sobralia* and *Sertifera*. The result is that there has been independent convergence to terminal inflorescences across both large clades in Sobralieae.

Flower size – There is a great range in flower size of Sobralieae. Species of *Elleanthus*, *Epilyna*, and *Sertifera* have relatively small flowers compared to the flowers of *Sobralia*. Variation in floral size is likely a consequence of shifts in pollination mode. The large flowers of *Sobralia* are mostly pollinated by large bees (e.g. *Eulaema*). The small flowers of

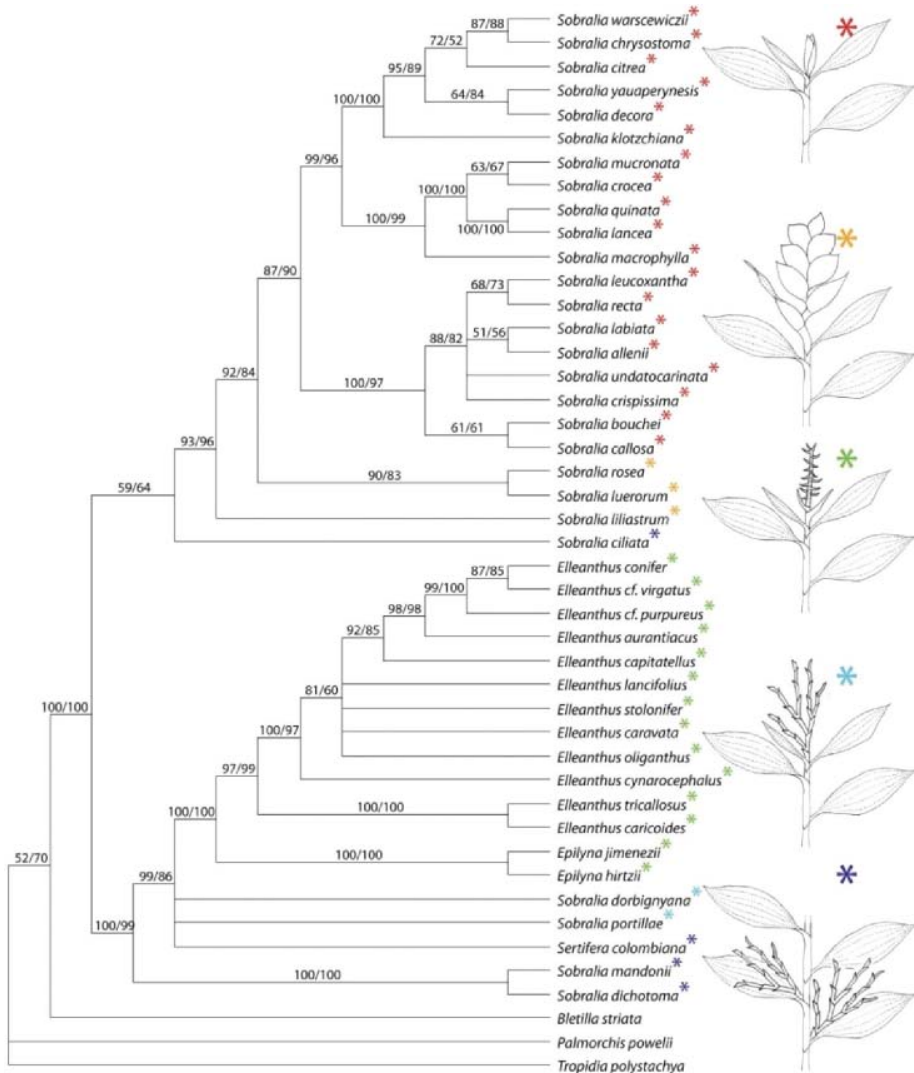


FIGURE 4. Bootstrap consensus tree of Sobralieae using all three DNA regions (ITS, *trnS-G*, and *ycf1*), to demonstrate relative support for clades. Numbers above or below branches indicate maximum likelihood and parsimony bootstrap percentages, respectively. Colored asterisks indicate distribution of major inflorescence morphology among taxa (*n.b.*, inflorescences are especially variable in *Elleanthus*, ranging from fractiflex to spiral and loosely racemose to capitate but are always terminal and consisting of a single axis as indicated by the illustration).

Elleanthus and *Sertifera* are usually pollinated by hummingbirds. However, pollinators of *Epilyna* and those of smaller, white-flowered species of *Elleanthus*, are unknown.

Variation of different pollinators and associated floral morphologies have been well documented in some systems (Thomson and Wilson, 2008). However, there are also taxonomic implications for shifts in pollination syndrome. Often, species

or groups of species that have shifted to a different syndrome have been traditionally placed in different genera. This nomenclatural bias to recognize genera because of variation in gross floral morphology has been demonstrated to conflict with phylogenetic relationships due to homoplasy in pollination-related floral characters. This bias is particularly apparent within *Sobralia*. *Sobralia callosa* has been segregated as *Lindsayella* Ames & C.Schweinf. because of its

distinctive hummingbird-floral syndrome, as opposed to the typical bee-floral syndrome that is characteristic of most species of *Sobralia*. However, the recognition of *Lindsayella* would elevate the degree of polyphyly in *Sobralia*. The floral morphology is misleading in this example because “distinctiveness” does not connote reciprocal monophyly.

In a larger phylogenetic context, relatively large flowers are plesiomorphic within the tribe, and generic concepts should not be based primarily on flower size. However, flower size combined with inflorescence position and structure are diagnostic, and we recommend that future generic circumscriptions be based on the combination of these apomorphic characters in conjunction with molecular data. Unfortunately, the type species of *Sobralia* is *S. dichotoma* (designated by Angely in *Fl. Analítica São Paulo* 6: 1268. 1973). This species does not belong to “core *Sobralia*” as defined in this paper. As a result of this quirk of history and because of the polyphyly of *Sobralia*, there are problematic nomenclatural issues with tribe Sobralieae. However, this problem is best resolved with more data and will be the subject of future research.

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EPIGENETIC INFORMATION – UNEXPLORED SOURCE OF NATURAL VARIATION

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ABSTRACT. Rapid progress is being made at the population-level in orchids, with a series of new molecular techniques being applied. One of the major problems observed in several groups of temperate orchids has been that ecologically distinct “taxa” do not appear to be genetically distinct. For example, we know that *Dactylorhiza traunsteineri*, *D. majalis*, and *D. ebudensis* are the products of hybridization between *D. fuchsii* and *D. incarnata*, but they have different ecologies and distributions within northwestern Europe. By comparing fingerprinting analyses of expressed regions to fingerprinting studies of methylation-sensitive sites in genomic DNA, we can detect patterns that indicate that some of these differences are due to changing epigenetic effects, which have been shown in several groups to be subject to environmental influence. Thus, taxa that are ecologically distinct but still appear genetically uniform may be the result of altered epigenetic controls of gene expression without any change in the underlying genetic material.

RESUMEN. Con la aplicación de nuevas técnicas moleculares, rápidos avances se han hecho para comprender la organización de las orquídeas a nivel de poblaciones. Uno de los mayores problemas encontrados en varios grupos de orquídeas de zonas templadas, ha sido que los taxa ecológicamente diferentes, al parecer son genéticamente similares. Por ejemplo, sabemos que *Dactylorhiza traunsteineri*, *D. majalis*, y *D. ebudensis* son resultado de la hibridización de *D. fuchsii* y *D. incarnata*, pero éstas tienen ecología y distribución diferentes dentro del nor-oeste de Europa. A través de la comparación de análisis “fingerprint” de regiones expresadas, con estudios “fingerprint” de sitios sensibles a la metilación en ADN genómico, podemos detectar patrones que indican que algunas de estas diferencias se deben a efectos epigenéticos, los cuales han sido encontrados en varios grupos que han sido expuestos a la influencia del ambiente. Así, taxa que son ecológicamente diferentes, pero que son al parecer genéticamente uniformes, podrían ser el resultado de la expresión de genes, debido a una alteración de los controles epigenéticos, pero sin que haya ocurrido ningún cambio en el material genético.

KEY WORDS: orchids, hybridization, fingerprinting analyses, epigeny, environmental influence

DNA sequences of all cells in an organism are essentially identical, but their transcription program is specific, depending on the cell type, developmental stage, age, location, etc. This critical feature of multicellular organisms is achieved through an array of epigenetic signals that are not encoded in the primary DNA sequence. Epigenetic signposts include DNA methylation (addition of a methyl group to a cytosine; Martienssen and Colot 2001), modifications of histones (core proteins that package DNA into chromosomal particles; Grewal & Moazed 2003), and small regulatory RNAs (Großhans & Filipowicz 2008). These signals control the activity of genes and

transposable elements in a relatively flexible manner (Rapp & Wendel 2005) by providing differential access to underlying genetic information to modulate the identity of cells.

In addition to the extensive involvement in developmental control and parent-of origin imprinted gene expression (Steimer *et al.* 2004, Henderson & Jacobsen 2007), epigenetic variants (epialleles) also control the organismic response to environmental conditions. Surprisingly, recent studies in various model organisms – including monozygotic twin humans (Fraga *et al.* 2005) – have indicated that some epialleles can be inherited

across generations through mechanisms that are not based on the primary DNA sequence. Accumulating evidence indicates that similarly to controlling cell differentiation within an organism, epigenetics can contribute, even in the absence of genetic or environmental heterogeneity, an additional layer of information that influences heritable phenotypic variation between individuals and evolutionary processes that act on this variation.

This may be particularly true for plants, where redundant copies of many genes are spread across the genomes as a result of ancient whole-genome duplication events (i.e., paleopolyploidization; Soltis *et al.* 2009) or individual gene duplication. A duplicated genetic background allows for increased levels of epigenetic variation that sorts out possible genetic variants. Moreover, epigenetic response mechanisms may be particularly important for immobile organisms, such as plants, that generally cannot respond within the same generation to environmental stimuli via relocation or behavioral alterations. Indeed, many examples of spontaneous heritable epialleles that demonstrably affect key phenotypic characters, such as pigmentation, floral shape, and pathogen resistance, have been discovered in flowering plants (Jablonka & Raz 2009), reflecting also the characteristically late partitioning of reproductive and vegetative cell lineages in higher plants.

Therefore, epigenetics could significantly improve our understanding of the natural mechanisms underlying phenotypic variation and the response of organisms to environmental change (Richards 2006, Bossdorf *et al.* 2008). However, epigenetic markers are at present only starting to be investigated in relation to evolutionary questions. We have little understanding regarding even basic questions, such as the extent and structure of epigenetic variation within and among natural populations. Recently, epigenetic markers have started to be used to investigate evolutionary questions related to stabilization of recently formed polyploids and evolutionary success. Several studies have provided evidence that epigenetic mechanisms activate dormant mobile elements and silence redundant genes immediately after polyploidization events, providing a flexible and reversible way of stabilizing cellular processes (see Paun *et al.* 2007, for a

review). However, little information is available on the stability of these epigenetic changes and their potential long-term evolutionary implications. We have studied a group of closely related polyploid species of orchids that developed during the last part of the Quaternary and found convincing evidence that epigenetic, rather than genetic, differentiation has been important for their diversification, driving ecological allopatry and reproductive isolation.

The case of *Dactylorhiza* allopolyploids

Dactylorhiza, a genus of temperate terrestrial orchids, has extensively evolved in a reticulate fashion resulting in significant but often subtle morphological and ecological variation that challenges species delimitation. Several *Dactylorhiza* allotetraploid ($2n = 80$) species have been repeatedly formed by hybridization between two broadly defined and geographically widespread parental lineages: the diploid ($2n = 40$) marsh-orchid, *D. incarnata* (L.) Soó *s.l.*, and the spotted orchid, *D. maculata* (L.) Soó *s.l.* (including, among others, diploid *D. fuchsii* (Druce) Soó; Heslop-Harrison 1968, Hedrén 1996, Hedrén *et al.* 2001, Pillon *et al.*, 2007, Hedrén *et al.* 2008). As a result, the taxonomy of many dactylorchids is widely considered complicated, causing long-standing controversies. The different sibling polyploid taxa can occur sympatrically, but they have different overall ecological requirements and distinct distributions. The full significance of the highly iterative polyploid evolution in this complex is not yet understood. It is intriguing that allotetraploid derivatives of the same progenitor pair can exhibit contrasting morphological and ecological properties that are maintained in spite of partly overlapping distributions.

Examples of such cases include European allotetraploids *D. majalis* (Rchb.) P.F.Hunt & Summerh. *s.str.*, *D. traunsteineri* (Saut. ex Rchb.) Soó *s.l.* (Fig. 1) and *D. ebudensis* (Wief. ex R.M.Bateman & Denholm) P.Delforge, each derived multiple times (except the last, a localized endemic, which has most probably a single origin) from unidirectional hybridization between diploids *D. fuchsii* (in all cases the maternal parent) and *D. incarnata* (Pillon *et al.*, 2007, and references therein). Combining the degree of concerted evolution in



FIGURE 1. The allotetraploid *Dactylorhiza traunsteineri* at a natural site in Yorkshire, Britain (Photo: O. Paun).

ITS alleles, and in agreement with the patterns of morphology and ecological preference, *D. majalis* is more derived and genetically homogeneous, inferred to be the oldest of the three allotetraploids and to have passed through glacially induced bottlenecks in southern Eurasia. It has a fairly wide ecological tolerance of soil moisture and occurs at present in damp meadows and fens in western and central Europe, the Baltic region, and northern Russia. In contrast, *D. traunsteineri* is a more recently evolved set of allotetraploids that is more heterogeneous and still maintains both parental ITS alleles (Pillon *et al.*, 2007). It probably originated post-glacially and at present shows a more localized and disjunct distribution in northwestern and central Europe (i.e., Britain, Scandinavia, and the Alps). It has narrow tolerances of both soil moisture and pH and grows in calcareous fens and marshes. A third allotetraploid, *D. ebudensis*, is a narrow endemic (at present forming a single population) in northwestern Scotland and may be as young as or younger than *D. traunsteineri*. The coastal dune habitat occupied

by *D. ebudensis* indicates its relatively narrow tolerances of both soil moisture and pH.

Despite ecological and morphological distinctiveness, neutral genetic differentiation between sibling *Dactylorhiza* allopolyploids, as studied with various molecular markers, including allozymes, ITS sequences, AFLPs, and plastid and nuclear microsatellites, has proved to be rarely detectable (Hedrén *et al.*, 2001; Pillon *et al.*, 2007). By contrast, a fingerprinting analyses of gene expression patterns (cDNA-AFLP; Paun *et al.*, 2007) resulted in a geographic structure (Paun *et al.*, 2010), grouping samples according to their provenance (i.e., Britain, Scandinavia, Alps, and Pyrenees). This may reflect the multiple independent origins of each tetraploid taxon (except *D. ebudensis*) or may provide evidence of local adaptation and/or stronger regional gene flow. However, several expression patterns have been found to be species-specific and clearly discriminate between the allopolyploid species (Paun *et al.*, 2010).

Moreover, epigenetic variation at loci spread across the genome, which was studied using methylation-sensitive amplified polymorphism (MSAP), clearly separates the three allopolyploids studied here (Fig. 2), in stark contrast to patterns of genome-wide genetic data (Hedrén *et al.*, 2001). The MSAP technique is similar to standard AFLP (Vos *et al.*, 1995) but uses two methylation-sensitive restriction isoschizomers (e.g., *MspI* and *HpaII*) as frequent cutters in parallel batches (Baurens *et al.*, 2003). The two isoschizomers recognize the same DNA sequence (5'-CCGG) but differ in their sensitivity to DNA methylation, so that *HpaII* and *MspI* do not recognize the restriction site when the internal, and respectively, external cytosine is methylated. Comparisons of the paired profiles for each individual allow precise assessment of methylation status at each restriction site.

Unexpectedly, methylation status analysis at 332 genome-wide MSAP markers resulted in species-specific patterns and confirm previous hypotheses of their evolutionary history (Heslop-Harrison, 1968; Pillon *et al.*, 2007). Indeed, the migration-induced genetic bottleneck triggered within *D. majalis* by the profound change of climatic conditions during the last glaciation seems to have homogenized methylation status among individuals and left only a

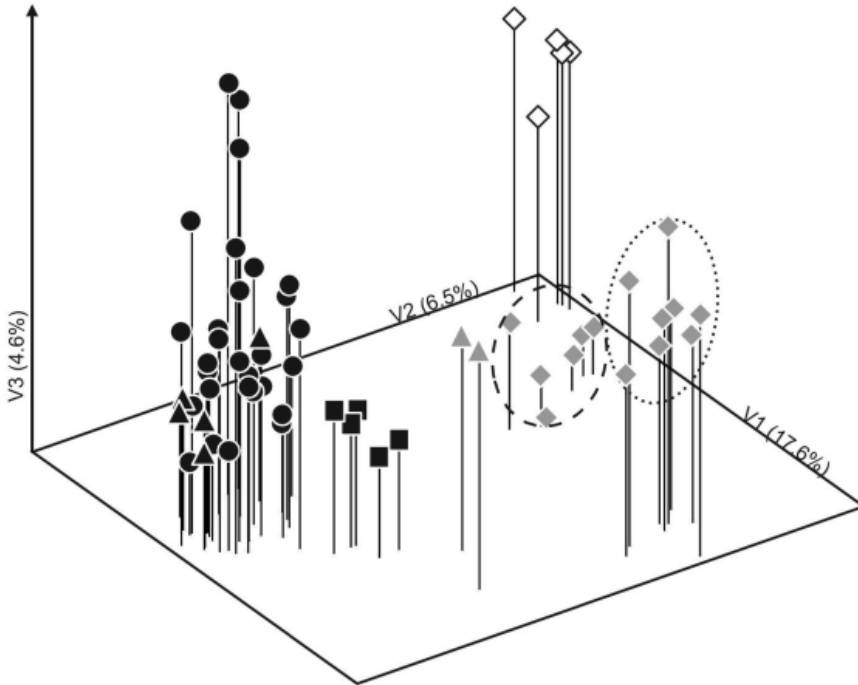


FIGURE 2. Principal coordinates analysis (goodness of fit 0.87 at $P = 0.001$) of methylation status of allotetraploid *D. majalis* (black symbols), *D. traunsteineri* (gray symbols), and *D. ebudensis* (open symbols). Geographical provenance is indicated by symbol shapes: squares, N. Pyrenees; diamonds, Britain; triangles, Scandinavia; circles, E. Alps. The dotted line encloses samples from Yorkshire, England, and the dashed line samples from N.W. Scotland.

weak within-species geographic differentiation (Fig. 2). As result of its postglacial formation and present disjunct distribution, the genome-wide methylation patterns of *D. traunsteineri* are more heterogeneous and correlate more clearly with geography.

Although containing information from both coding and non-coding DNA regions, the methylation markers studied reflect better gene-expression differences in the three allopolyploid siblings than neutral genetic data (Mantel tests, $r = 0.81$ vs. $r = 0.67$ both significant at $P < 0.01$). Therefore, it seems that habitat preference shapes similar expression patterns in some, but not all, of the independent allopolyploidization events in this group, operating via epigenetic regulatory effects under environmental influence rather than via sequence (genetic) divergence (Paun *et al.*, in press).

To test further our hypothesis of adaptation through selection of epigenetic variants, we have performed scans for outlier epigenetic loci, looking for adaptive epiloci that have been shaped by

natural selection. We have used two complementary approaches: 1) multiple univariate logistic regressions between epigenetic data and eco-climatic variables, performed using SAM (Joost *et al.*, 2007); and 2) a Bayesian outlier locus approach as implemented in BayeScan (Foll and Gaggiotti, 2008). The latter approach estimates the posterior probability of each locus being under selection, and it is able to differentiate the type of selection each marker was subjected to (divergent vs. purifying). Within our methylation data, BayeScan identified 23 epiloci as being under divergent selection; SAM pinpointed 14 methylation markers as being adaptive, and ten epiloci were identified by both approaches. For most of the outlier epiloci, their presence-absence patterns correlated with water availability, which seems to be a key ecological factor in driving environmental allopatry within the three allopolyploid species. If maintained over evolutionary timescales, environmental allopatry may effectively limit dispersal between populations,

thus promoting divergence via the stochastic effects of drift (Nosil *et al.*, 2009) and/or further selection. Therefore, epigenetic regulatory processes play a clear role in incipient adaptation and evolution of the allopolyploid *Dactylorhiza* species by influencing primary phenotypic diversity at the interface between genetics and the environment. The fact that recent evolutionary history is discernible in epigenetic patterns indicates that such markers could be used for investigations in closely related, rapidly radiating groups when genetic markers may fail to provide relevant information.

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OF GREENISH *ENCYCLIA*: NATURAL VARIATION, TAXONOMY, CLEISTOGAMY, AND A COMMENT ON DNA BARCODING

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ABSTRACT. The species-level taxonomy of *Encyclia* has been disputed considerably because of the great morphological similarity among many of the taxa, particularly in the complex of species related to *E. chloroleuca* and *E. gravida*, characterized by small, greenish flowers. Current phylogenetic results are insufficient to assess the natural lineages of the greenish species of *Encyclia*, and species concepts in this group are discussed here independently from previous schemes of classification and current nomenclatural uses. In Mesoamerica, traditional taxonomic approaches shifted from broad views of species circumscriptions to the recognition of a large number of finely split taxa. However, the relative paucity of specimens available for study led both approaches to fail to appreciate the range of natural variation, with the consequence of nomenclatural inflation and misunderstanding of species diversity. On the basis of a better sample, we reduce the supposedly rare and variable *E. amanda* to synonymy of *E. chloroleuca* and discuss the case of cleistogamous individuals of *Encyclia* referred to *E. gravida*. On the basis of floral morphology, we suggest that the few documented records of *E. gravida* may simply represent self-pollinating forms belonging to different taxa.

RESUMEN. La necesidad de conceptos específicos delimitados rigurosamente ha sido enfatizada para contar con inventarios de biodiversidad más certeros. Sin embargo, mientras el número de nuevos organismos descritos crece con un ritmo que no tiene antecedentes, nuestros conocimientos de mucho de los antiguos y nuevos taxones está todavía basado en un número extremadamente reducido de muestras, con una apreciación en el mejor de los casos reducida de su variación natural. Aun cuando se consideren en su circunscripción más estrecha, cercana al concepto original propuesto por Hooker (es decir, plantas con pseudobulbos piriformes, una inflorescencia terminal que no nace de una espata, hojas coriáceas a carnosas, un labio membranáceo generalmente provisto de lóbulos laterales bien desarrollados que envuelven la columna, y un callo forcipato en el disco del labelo), las “verdaderas” especies de *Encyclia* siguen siendo un grupo taxonómico difícil. Esto es particularmente evidente para un amplio complejo de especies emparentadas con *E. chloroleuca*, caracterizadas por flores pequeñas y en su mayoría verdosas, que se distribuyen en todo el Neotrópico. Las interpretaciones taxonómicas tradicionales de este grupo en la región mesoamericana oscilan entre el reconocimiento de 13 taxones finamente partidos y la taxonomía más conservadora de Ames y sus colegas en Harvard, quienes adoptaron conceptos amplios y en el grupo reconocieron solamente cuatro especies (con cinco subespecies). Ambas interpretaciones, sin embargo, se basaron fundamentalmente en el estudio de material escaso, a menudo unas pocas colecciones por cada país, según un sistema que ha prevenido la apreciación de la variación natural y últimamente llevó los taxónomos hacia una inflación en la nomenclatura o, alternativamente, a un concepto generalmente equivocado de la diversidad de las especies. En Mesoamérica, hoy en día reconocemos nueve especies de *Encyclia* verdosas (una de ellas probablemente no descrita) algunas de las cuales se extienden a Suramérica. La taxonomía de los taxones Andinos de este grupo, sin embargo, necesita urgentemente de revisión. En los últimos años, la secuenciación del genoma de las plantas, sobretodo a través de los códigos de barras, se ha reconocido como una poderosa herramienta para evaluar la congruencia de los conceptos específicos y descubrir taxones crípticos difíciles de separar con métodos taxonómicos tradicionales. Sin embargo, los códigos de barras por si solos nunca son suficiente para describir una nueva especie, aunque a un cierto punto códigos muy claramente divergentes

pueden utilizarse (con otros conjuntos de datos) como base para decisiones nomenclatoriales. Para priorizar cuales “morfoespecies” y cuales individuos deben ser secuenciados en complejos de especies problemáticos, el análisis del rango de variación intra- e interespecífico constituye un primer paso necesario para ubicar los códigos de barras en el contexto de un acercamiento taxonómico complejo, que finalmente utilice las técnicas basadas en el ADN en conjunto con otras disciplinas, tales como la filogeografía, la morfología comparativa, la genética de poblaciones, la ecología, el desarrollo y la biología reproductiva, para delimitar de forma mejor las unidades de la diversidad de orquídeas.

KEY WORDS: Orchidaceae, Laeliinae, *Encyclia*, Central America, taxonomy, cleistogamy

The need for rigorously delimited species concepts has been emphasized for the accuracy of biodiversity inventories. However, while the number of newly described organisms increases at an unprecedented rate, our knowledge of many of the old and new taxa is still based on a minimal number of samples. This is particularly evident in the case of tropical orchids, an impressively diverse group of organisms, which are in many cases only fragmentarily known through a few records randomly collected over their geographic ranges. The proliferation of synonyms in species descriptions, which traditionally plagued orchid taxonomy, is largely due to the difficulty of appreciating the range of natural variation among and between populations on the basis of the limited available samples, particularly in the case of taxa with broad distributions. Before the advent of large floristic projects such as *Flora Neotropica* in the 1960s (to date, only the genera *Bulbophyllum*, *Epidendrum*, *Isochilus*, *Mormodes*, and *Vanilla* have been scheduled for inclusion) and *Flora Mesoamericana* in the last 15 years, floristics have been published mostly on a national or local scale, and widespread taxa have often received a different name in each of the countries where they naturally occur.

In the case of the Neotropical orchid genus *Encyclia* Hook., the interest of systematic botanists mostly focused in the past on a more convenient - or more natural - way to circumscribe the genus (e.g., Lindley 1842, 1853, Ames *et al.* 1936, Dressler, 1961, 1984, Dressler & Pollard 1971, 1974, Withner 1998, 2001, van den Berg *et al.* 2000, Higgins 1998, Higgins *et al.* 2003), but the task of describing the diversity of *Encyclia* was essentially achieved in a floristic context with little or no attention given to any scheme of relationships among species. Joseph D. Hooker established *Encyclia* in 1828 based on a species native to Rio de Janeiro, Brazil, and separated

it from “the splendid species of *Cattleya*,” at that time including only three species (Hooker 1828). Hooker’s *Encyclia viridiflora*, the type species for the genus, is indeed a peculiar and poorly known plant with many unique characteristics compared to its close relatives. These include non-resupinate flowers, a short column, the lateral lobes of the lip almost completely fused with the midlobe, and a nonforcipate basal callus (Hooker, 1828, Withner 1996; Fig. 1). By the midpoint of the 19th century, under the authority of John Lindley, the genus was merged under *Epidendrum* L., and the type species was assigned to



Figure 1. Original illustration of *Encyclia viridiflora*, from Curtis’s *Botanical Magazine* 1828, pl. 2851.

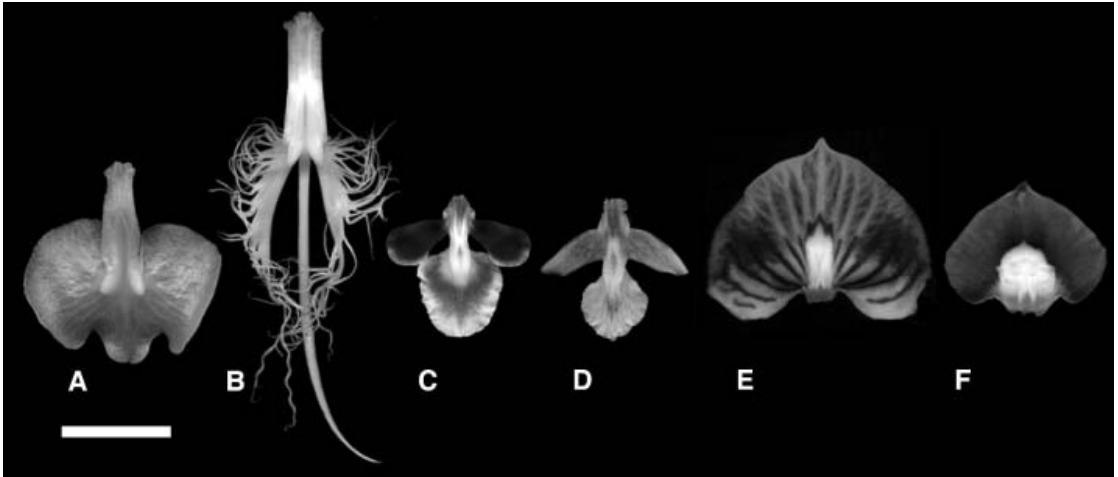


FIGURE 2. Labella of *Epidendrum*, *Encyclia* and *Prosthechea* species. A - *Epidendrum storkii*; B - *E. ciliare*; C - *Encycliamooreana*; D - *E. chloroleuca*; E - *Prosthechea cochleata*; F - *P. spondiada*. Scale bar = 1 cm.

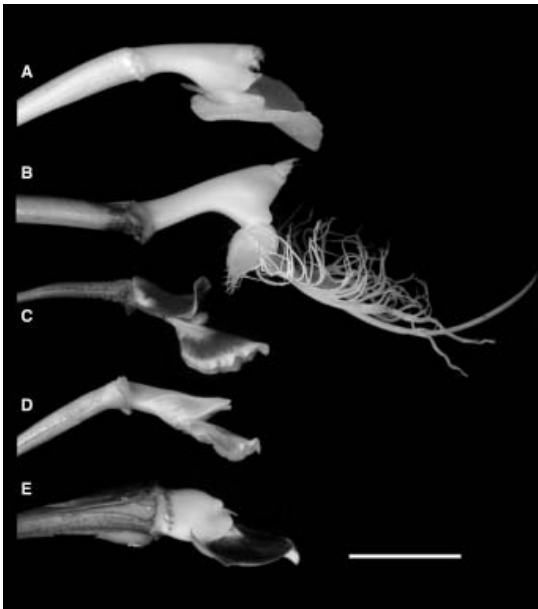


FIGURE 3. Lateral views of the gynostemium and labella of *Epidendrum*, *Encyclia* and *Prosthechea* species, showing various degrees of adnation between column and lip. A — *Epidendrum storkii*; B - *E. ciliare*; C - *Encycliamooreana*; D - *E. chloroleuca*; E - *Prosthechea spondiada*. Scale bar = 1 cm.

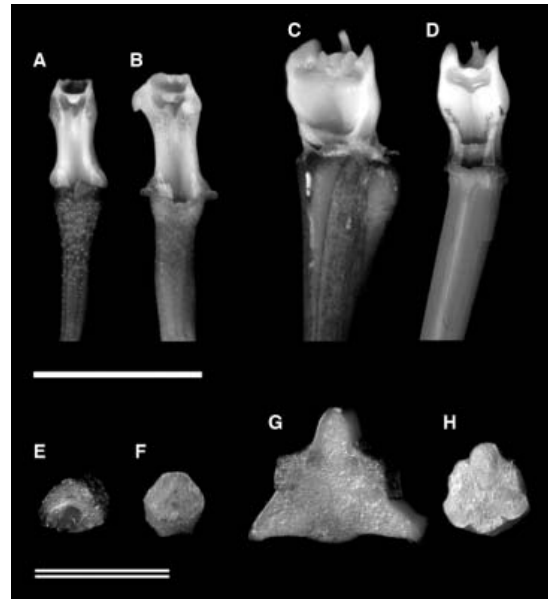


FIGURE 4. Columns and sections through the ovaries of *Epidendrum*, *Encyclia* and *Prosthechea* species. A-D, Columns. A - *Encycliamooreana*; B - *E. chloroleuca*; C - *Prosthechea spondiada*; D - *P. cochleata*; E-H, Transverse sections through the ovaries. E - *Encycliamooreana*; F - *E. chloroleuca*; G - *Prosthechea spondiada*; H - *P. cochleata*. Scale bar = 1 cm; double bar = 5 mm.

E. subgenus *Encyclium* Lindl. section *Hymenochyla* Lindl. (Lindley 1831, 1842, 1853). Despite attempts to revive *Encyclia* by Rudolf Schlechter (1914, 1918, 1922, 1923), it was not until 1961 when Robert L. Dressler offered conclusive evidence to recognize the

distinctness of the genus and the need to segregate it from *Epidendrum* (Dressler 1961; Fig. 2-3). However, even when the genus is considered in its narrower circumscription approximating the original concept by Hooker (Withner 1996), today widely

accepted notwithstanding the removal of the large bulk of species now assigned to *Prosthechea* Knowl. & Westc. (Higgins 1998; Fig. 4), the 'true' *Encyclia* species still form a difficult taxonomic group, and the identity of many of the species in the genus remains confused (see, for example, Dressler 2004 vs. Withner 1998, 2001).

The taxonomy of greenish *Encyclia*

Species-level taxonomy of *Encyclia* has been widely disputed, largely because of the great morphological similarity among many of the taxa — particularly in the large complex of species related to *E. chloroleuca* and *E. gravida* (Lindl.) Schltr., which are characterized by small and mostly greenish flowers — and the application of old names often based on poorly defined species concepts. We refer to these complexes here by the collective name of 'greenish encyclias'. 'Green' is not in any way a taxonomic category, and current phylogenetic results are insufficient to assess the natural lineages of the greenish species, so we feel free to discuss this group independently from any previous scheme of classification and current nomenclatural uses.

With several exceptions, which are easy to identify by unique sets of floral and vegetative features (such as the distinctive *E. adenocaula* (Llave & Lex.) Schltr., *E. cordigera* (Kunth) Dressler, or *E. phoenicea* (Lindl.) Neumann), species of *Encyclia* commonly share a common, uniform, and generalized morphological scheme. The plants are provided with ovoid pseudobulbs that bear two or three (rarely four) coriaceous leaves at the apex; inflorescences are frequently paniculate and variable in length even at different stages of the same individual, with the rachis and the pedicellate ovaries variously verruculose; flowers have three similar sepals, frequently clawed petals, a distinctly trilobed lip with the lateral lobes erect and the midlobe variously adorned with thickened veins, which in many taxa appear as longitudinal-radiating, sometimes prominent keels, and a basal, forcipate callus; the column is normally straight and provided with apical wings, which commonly embrace the isthmus that separate the lateral lobes from the midlobe. The perianth parts are frequently greenish or pale tan, with the lip cream-white, often provided with rose-purple

stripes along the main veins. Populations of *Encyclia* corresponding to this scheme are broadly distributed in the Neotropics, from Florida, Mexico, and the West Indies, throughout Central America, to Argentina and Paraguay, occurring both in Andean and Amazonian South America (Table 1).

For plants located in Mesoamerica, which we have investigated more closely during the past few years, traditional taxonomic approaches to understanding *Encyclia* have shifted from broad views of species circumscriptions that considered many of the described species of *Epidendrum* and *Encyclia* from Mesoamerica as a single polymorphic alliance (Ames *et al.*, 1935, 1936) to the recognition of a large number of finely split taxa (Withner, 1996). According to the different taxonomic treatments, the group of greenish Central American *Encyclia* includes 4 to 13 species. The use of broad concepts to avoid the difficulty of finding clear ways to distinguish between closely allied species reached its apex during the first half of the last century with the work by Oakes Ames and his co-workers at Harvard University. In their studies on the genus *Epidendrum* (including *Encyclia*) of North and Central America (Ames *et al.*, 1936), they considered most of the species with medium-sized flowers and an orbicular midlobe of the lip as varieties of a single variable species, a broadly defined *Epidendrum oncidoides* Lindl. However, scrutiny of more material now available proved that this gross lumping of Central American species under a few of the oldest names is untenable (Dressler and Pollard, 1976; Withner, 1996; Dressler, 2004; Pupulin, 2005, 2006). At the same time, a finely split concept for this group is equally untenable. The relative paucity of specimens available for study, together with the adoption of unconfirmed geographical records and the pronounced polymorphism of many Central American species, induced some botanists to adopt an excessively fine approach, often relying on subtle differences in type specimens that do not hold up in comparison with broader series of records (Withner, 1998, 2001). In both approaches, the impossibility of fully appreciating the range of natural variation eventually drove taxonomists to nomenclatural inflation or, alternatively, to a general misunderstanding of species diversity.

TABLE 1. Described species of *Encyclia* with greenish flowers.

Species	Distribution
<i>Encyclia acuta</i> Schltr.	Brazil, Venezuela
<i>Encyclia acutifolia</i> (Sw.) Nir	Jamaica
<i>Encyclia alanjensis</i> (Ames) Carnevali & Romero	Panama
<i>Encyclia alboxanthina</i> Fowlie	Brazil
<i>Encyclia amanda</i> (Ames) Dressler	Panama
<i>Encyclia amicta</i> (Lind. & Rchb.f.) Schltr.	Bolivia, Brazil, Paraguay, Peru, Venezuela
<i>Encyclia angustiloba</i> Schltr.	Bolivia, Ecuador, Peru
<i>Encyclia argentinensis</i> (Spegazzini) Hoehne	Argentina, Brazil, Paraguay
<i>Encyclia aspera</i> (Lindl.) Schltr.	Colombia, Ecuador, Peru
<i>Encyclia asperirachis</i> Garay	Colombia
<i>Encyclia asperula</i> Dressler & Pollard	Mexico, Guatemala, Belize
<i>Encyclia bradfordii</i> (Griseb.) Carnevali & Ramírez	Tobago, Trinidad, Venezuela
<i>Encyclia brenesii</i> Schltr.	Costa Rica
<i>Encyclia ceratistes</i> (Lindl.) Schltr.	El Salvador to Colombia and Venezuela
<i>Encyclia chloroleuca</i> (Hook.) Neumann	Belize to Brazil and Peru
<i>Encyclia confusa</i> Menezes	Brazil
<i>Encyclia davidhuntii</i> Withner & de Fuente	Belize, Guatemala, Honduras, Costa Rica
<i>Encyclia diurna</i> (Jacq.) Schltr.	Colombia, Suriname, Venezuela
<i>Encyclia expansa</i> (Rchb.f.) Ortíz	Colombia
<i>Encyclia fehlingii</i> (Sauleda) Sauleda & Adams	Bahamas
<i>Encyclia flava</i> (Lindl.) Porto & Brade	Brazil, Venezuela
<i>Encyclia fucata</i> (Lindl.) Britt. & Millsp.	Bahamas, Cuba
<i>Encyclia glandulosa</i> (Kunth) Ortíz	Colombia, Venezuela
<i>Encyclia gonzalensis</i> Menezes	Brazil
<i>Encyclia goyazensis</i> Menezes ex Fowlie	Brazil
<i>Encyclia granitica</i> (Lindl.) Schltr. Venezuela,	Guyana, Suriname, Brazil
<i>Encyclia guentheriana</i> (Kränzl.) R. Vásquez	Bolivia
<i>Encyclia guyanensis</i> Carnevali & Romero	Venezuela, Guyana, Suriname
<i>Encyclia hunteriana</i> Schltr.	Panama
<i>Encyclia inaguensis</i> Nash ex Britt. & Millsp.	Bahamas, Turks and Caicos Islands
<i>Encyclia leucantha</i> Schltr.	Colombia, Venezuela
<i>Encyclia lineariloba</i> Withner	Mexico, Guatemala, Nicaragua
<i>Encyclia linearifolioides</i> (Kränzl.) Hoehne	Brazil
<i>Encyclia longifolia</i> (Barb.Rodr.) Schltr.	Brazil
<i>Encyclia maderoi</i> Schltr.	Colombia
<i>Encyclia maravalensis</i> Withner	Trinidad
<i>Encyclia monticola</i> (Fawc. & Rendle) Acuña	Cuba, Hispaniola, Jamaica, Trinidad
<i>Encyclia mooreana</i> (Rolfe) Schltr.	El Salvador to Panama
<i>Encyclia mapiriensis</i> Kränzl.	Brazil
<i>Encyclia naranjapatensis</i> Dodson	Ecuador
<i>Encyclia nematocaulon</i> (A.Rich.) Acuña	Mexico to Nicaragua, and Cuba
<i>Encyclia odoratissima</i> (Lindl.) Schltr.	Brazil
<i>Encyclia oncidioides</i> (Lindl.) Schltr.	Brazil
<i>Encyclia ossenbachiana</i> Pupulin	Costa Rica
<i>Encyclia pachyantha</i> (Lindl.) Hoehne	Venezuela, French Guyana, Guyana, Brazil
<i>Encyclia patens</i> Hooker	Brazil
<i>Encyclia pauciflora</i> (Barb.Rodr.)	Porto & Brade Brazil
<i>Encyclia pedra-azulensis</i> Menezes	Brazil
<i>Encyclia peraltensis</i> (Ames) Withner	Costa Rica
<i>Encyclia picta</i> (Lindl.) Hoehne	Guyana

TABLE 1. *Continues.*

Species	Distribution
<i>Encyclia piracanjubensis</i> Menezes	Brazil
<i>Encyclia porrecta</i> B.R.Adams & P.J.Cribb	Belize
<i>Encyclia powellii</i> Schltr.	Panama
<i>Encyclia purpusii</i> Schltr.	Mexico
<i>Encyclia recurvata</i> Schltr.	Venezuela to Brazil
<i>Encyclia saltensis</i> Hoehne	Brazil
<i>Encyclia sclerocladia</i> (Lindl. ex Rchb.f.) Hoehne	Peru
<i>Encyclia steinbachii</i> Schltr.	Bolivia
<i>Encyclia stellata</i> (Lindl.) Schltr.	Costa Rica to Colombia and Venezuela
<i>Encyclia tampensis</i> (Lindl.) Small	Florida, Bahamas
<i>Encyclia tarumana</i> Schltr.	Brazil
<i>Encyclia thrombodes</i> (Rchb.f.) Schltr.	Bolivia, Peru, Brazil
<i>Encyclia tonduziana</i> Schltr.	Costa Rica
<i>Encyclia trachypus</i> Schltr.	Ecuador
<i>Encyclia triangulifera</i> (Rchb.f.) Acuña	Cuba
<i>Encyclia tripartita</i> (Vell.) Hoehne	Brazil
<i>Encyclia tuerckheimii</i> Schltr.	Mexico to Nicaragua
<i>Encyclia viridiflava</i> Menezes	Brazil
<i>Encyclia wagneri</i> Schltr.	Venezuela
<i>Encyclia xerophytica</i> Pabst.	Brazil
<i>Encyclia xypheres</i> (Rchb.f.) Schltr.	Mexico
<i>Encyclia yauaperyensis</i> (Vell.) Hoehne	Bolivia, Brazil
<i>Encyclia yucatanense</i> Schltr.	Mexico
<i>Epidendrum affine</i> Focke	Venezuela
<i>Epidendrum chloranthum</i> Lindl.	—
<i>Epidendrum halatum</i> Garay & Dunsterv.	Venezuela
<i>Epidendrum glutinosum</i> Scheidw.	Brazil
<i>Epidendrum ramonense</i> Rchb.f.	Costa Rica
<i>Epidendrum serronianum</i> Barb.Rodr.	Brazil
<i>Epidendrum spectabile</i> Focke	Venezuela

Size (of the sample) matters

One should emphasize that more common species, even in the group of the greenish *Encyclia*, are usually less taxonomically problematic. Taxonomy based on morphological analysis still relies on the interpretation of putative disjunctions along continuous variations of features, but this requires a representation of variation in character states as large as possible. In the Mesoamerican region, the ample records available for species such as *Encyclia ceratistes* (Fig. 5) or *E. mooreana* (Fig. 6) allow botanists not only to understand variation better, adopting sharper species circumscriptions and correctly interpreting heterotypic synonyms, but also detect those sister species that do not fit well any of the known specific patterns (Pupulin, 2006; Table 2).

A particularly critical situation is posed by taxa that span great geographic areas. As previously noted, floristic treatments have been mostly published on a national scale, and species comparison has been in many cases limited to the taxa recorded in immediately adjacent countries. Due to their overall diversity, the orchid floras of Central and South America have seldom been critically compared. It is a normal assumption that the patterns of geographic distribution in the Orchidaceae are generally defined by major continental masses, and the lack of generalist orchidologists has prevented in-depth monographic work spanning the whole Neotropics. When preparing a revision of *Encyclia* for the flora of Costa Rica (Pupulin and Bogarin, in preparation), we found one of these cases. Before the intensive field activity aimed at obtaining specimens for this study, *E. amanda* was known only

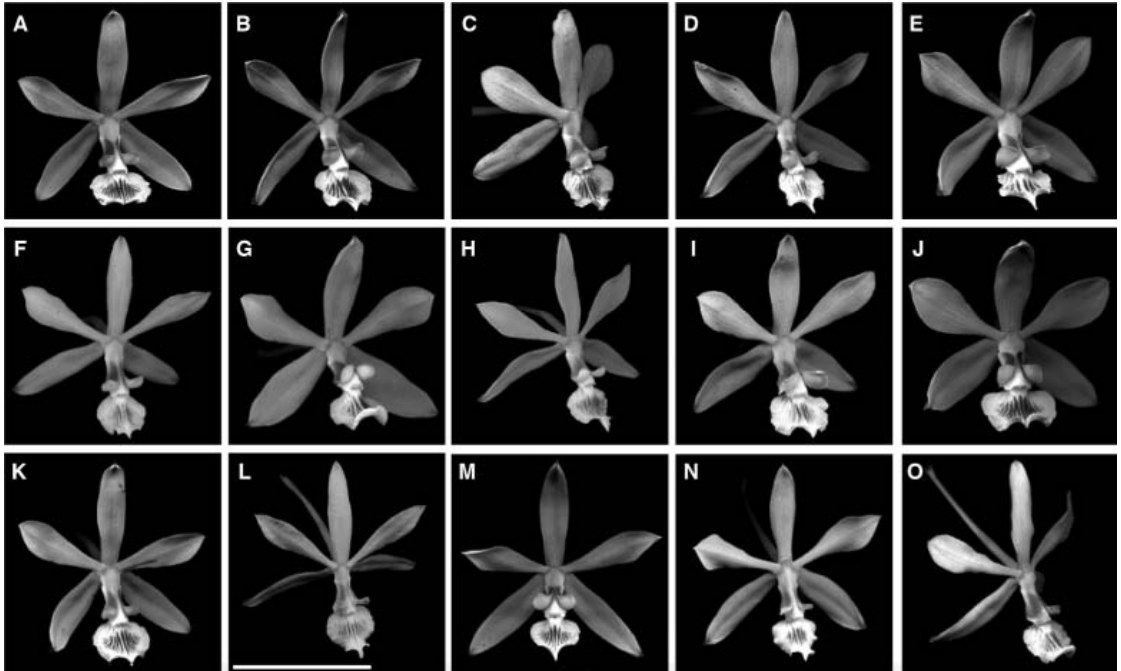


FIGURE 5. Floral variation in *Encyclia ceratistes*. A - Bogarín 3800; B - Bogarín 3799; C - Bogarín 3896; D - Bogarín 3797; E - Bogarín 3798; F - Pupulin 5641; G - JBL-s.n.; H - Pupulin 5200; I - Bogarín 5520; J - Bogarín 3806; K - Bogarín 3805; L - Pupulin 5303; M - Bogarín 3803; N - Pupulin 5641; O - Bogarín 3802. All the vouchers at JBL-Spirit. Scale bar = 2 cm.

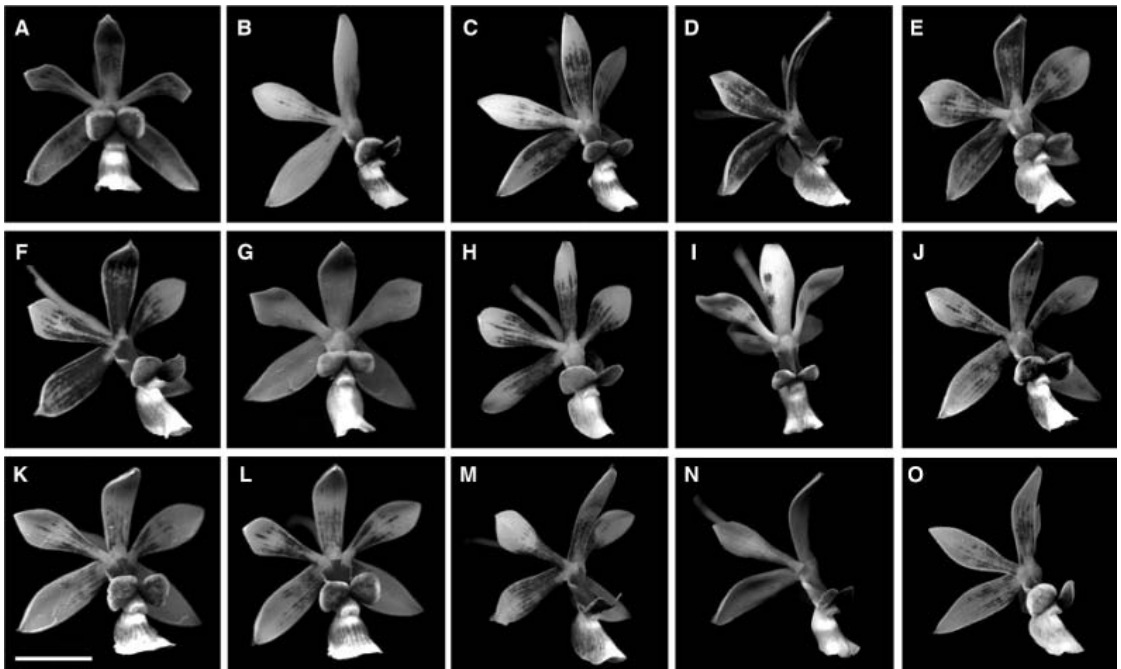


FIGURE 6. Floral variation in *Encyclia mooreana*. A - Bogarín 3810; B - JBL-06301; C - Bogarín 3721; D - JBL-08705; E - Bogarín 3787; F - Karremans 1356; G - JBL-08701; H - Gómez 3; I - Bogarín 3792; J - JBL-08704; K - Bogarín 3790; L - Bogarín 3791; M - JBL-08708; N - JBL-10044; O - JBL-08707. All the vouchers at JBL-Spirit. Scale bar = 1 cm.

TABLE 2. Comparison between taxonomic classifications of Costa Rican greenish *Encyclia*.

Williams 1946	Dressler & Pollard 1974	Dressler 1983	Withner 1998 & ss.	Dressler 2003	Pupulin & Bogarín (in prep.)
<i>Epidendrum oncioides</i> var. <i>gravidum</i>	<i>Encyclia gravida</i>	<i>Encyclia amanda</i>	<i>Encyclia amanda</i>		<i>Encyclia chloroleuca</i>
	<i>Encyclia alanjensis</i>		<i>Encyclia davidhunthii</i>		
	<i>Encyclia hunteriana</i>	<i>Encyclia gravida</i>	<i>Encyclia gravida</i>	<i>Encyclia stellata</i>	<i>Encyclia gravida</i> <i>Encyclia stellata</i>
			<i>Encyclia peraltensis</i>	<i>Encyclia peraltensis</i>	<i>Encyclia peraltensis</i>
<i>Epidendrum oncioides</i> var. <i>ramonense</i>	<i>Encyclia ceratistes</i>	<i>Encyclia ceratistes</i>	<i>Encyclia ceratistes</i>	<i>Encyclia ceratistes</i>	<i>Encyclia ceratistes</i>
<i>Epidendrum oncioides</i> var. <i>mooreanum</i>		<i>Encyclia mooreana</i>	<i>Encyclia mooreana</i>	<i>Encyclia mooreana</i>	<i>Encyclia mooreana</i>
	<i>Encyclia tuerckheimii</i>	<i>Encyclia tuerckheimii</i>	<i>Encyclia tuerckheimii</i>		
					<i>Encyclia ossenbachiana</i>

from a limited number of specimens. Withner (1998) mentioned no more than eight collections in the world's herbaria and included Costa Rica in the species distribution with no specimen citation (Withner, 2001). On the basis of the studied records, he characterized the species by the few-flowered (4 or 5), inflorescences that barely surpass the length of the leaves. Our collections revealed that this taxon is common in Costa Rica (Fig. 7) but extremely variable both in plant architecture and floral morphology. Well-developed inflorescences are commonly paniculate, many-flowered (12-30) and 60-70 cm long, but juvenile specimens may flower with short, simple, few-flowered racemes to only 15 cm long. Flowers vary greatly both within and among populations, and cultivated specimens show that floral variation may be notorious even on the same specimen at different flowering times (Fig. 8). When this range of variation is taken in account, the common Costa Rican taxon is not distinguishable from South American specimens of *Encyclia chloroleuca* (which has nomenclatural priority), and the study of the type specimen of *Epidendrum chloroleucum* at Kew confirmed our suspicions (Fig. 9). However, on the basis of the materials at our disposition, it is quite possible that the name *E. amanda* should be maintained for a distinct species from Panama and, perhaps, northern Colombia. The taxonomy of the Andean and Brazilian taxa in the group is still in urgent need of revision, and we cannot judge at this point how many specific epithets from these regions should be reduced to synonymy with *E. chloroleuca*.

Cleistogamous *Encyclia*

Even more intriguing and taxonomically difficult are those cases in which materials are hard to find due to the intrinsic nature of the relevant species. Now that we are concluding our revision of Costa Rican *Encyclia*, we will accept the concept of *E. gravida* with some reservation concerning the application of the name and the real identity of this taxon. Lindley (1849) originally described *Epidendrum gravidum* on the basis of a Mexican collection by Hartweg (*Hartweg s.n.*, the type!). The holotype at Kew (Fig. 10) consists only of a single inflorescence originally bearing four fruits (one of which was removed in 1923 and sent to Oakes Ames for study); the remaining sepals measure 10-11 mm in length and ca. 3 mm in width (Lindley, 1849; see also Carnevali *et al.*, 1994). Lindley (1849) considered it a "mere botanical curiosity," and in the protologue he gave no information about the vegetative characters of the new taxon. Florally, it was characterized by the linear lateral lobes of the lip, the ovate, acute midlobe provided with thickened veins, and the column with stigmatic, inflexed wings. After the original description, the name has been mostly adopted to identify populations of *E. stellata*, which shares with *E. gravida* a distinctly papillate ovary and the presence of prominent veins on the lip, but in a few cases the material referred to seems to match the original concept of Lindley.

Cleistogamy is a breeding system defined as the production of permanently closed, self-pollinated

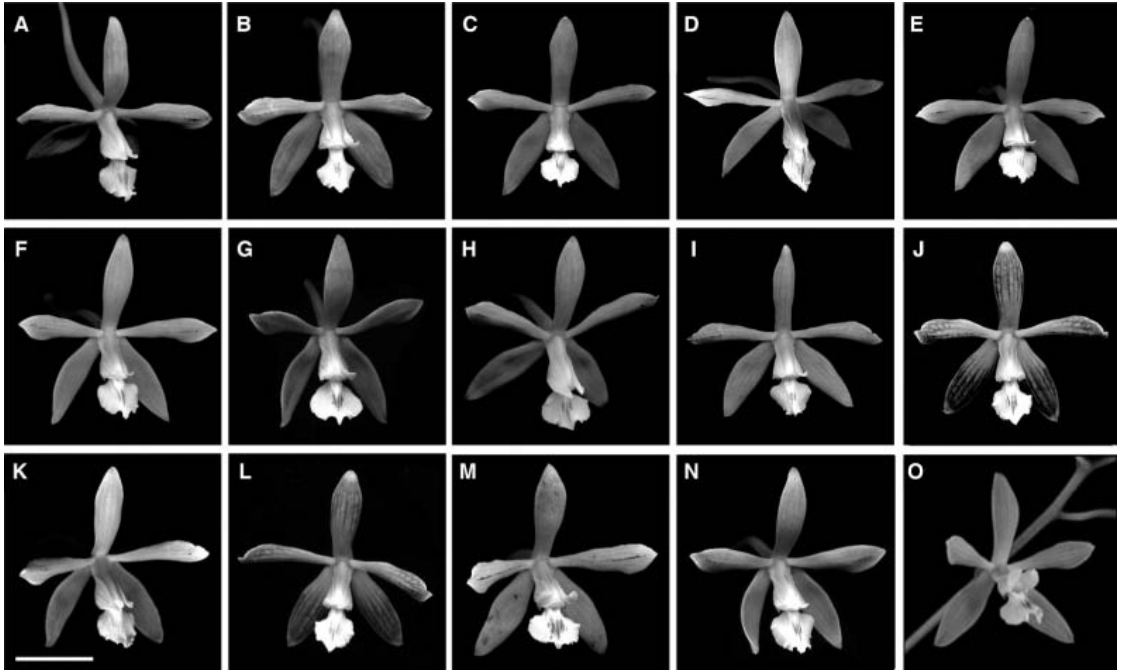


FIGURE 7. Floral variation in *Encyclia chloroleuca*. A - Bogarín 2537; B - Bogarín 2532; C - Bogarín 2537; D - Bogarín 3111; E - Bogarín 2544; F - Pupulin 3043; G - Ossenbach 365; H - Bogarín 2537; I - Bogarín 2544; J - Pupulin 3045; K - Pupulin 6536; L - Pupulin 3045; M - Pupulin 3045; N - Pupulin 3043; O - Pupulin 3044. All the vouchers at JBL-Spirit. Scale bar = 1 cm.

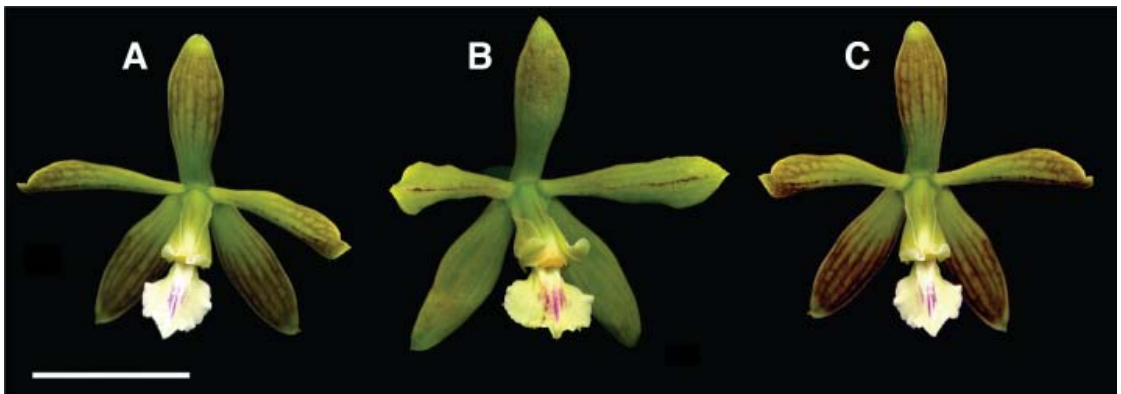


FIGURE 8. Floral variation in a single specimen of *Encyclia chloroleuca*. Pupulin 3045, flowering on 26 January 2003 (A), 11 June 2003 (B), and 3 March 2004 (C). Vouchers at JBL-Spirit. Scale bar = 1 cm.

flowers, and it is now recognized as an important system found in a variety of plant taxa (Culley and Klooster, 2007). Fertilization within cleistogamous flowers occurs without the intervention of pollinators, and in the Orchidaceae this is usually accomplished by direct transfer of pollen grains from anther to stigma. In cleistogamous species, selfpollinating flowers may

be the only type produced, but they may also appear together on the same plant along with typically insect-pollinated flowers. Complete cleistogamy, defined as the production of only cleistogamous flowers on an individual, has been especially reported in orchid species, but most indications are based on observations of only a few individuals.

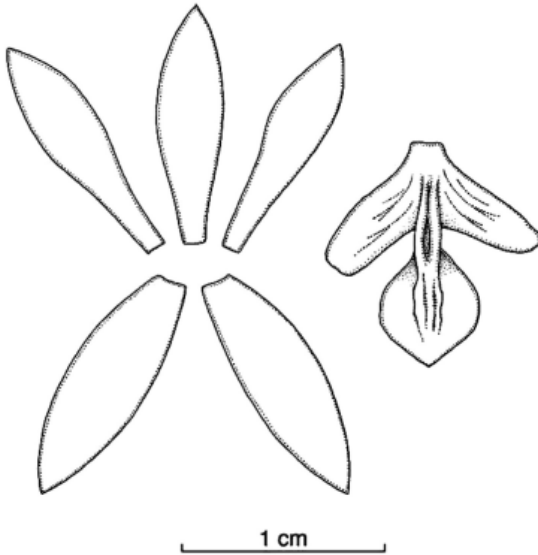


FIGURE 9. Tracing of a flower from the holotype of *Epidendrum chloroleucum*. Drawn with the aid of a camera lucida by D. Bogarín.

There are obvious difficulties in documenting “true” cleistogamous *Encyclia* individuals. Plants of other species related to “*E. grvida*” are frequently pollinated in the wild, and the pollination rate is high for several taxa. In Costa Rica, where large populations of *E. ceratistes* are found in pristine and altered habitats, it is not uncommon to observe inflorescences bearing literally tens of fruits. As these individuals are vegetatively indistinguishable from cleistogamous forms, the only way to tell the two entities apart is a careful observation of the perianth parts. In true cleistogamous specimens, the ovary begins to swell and elongate while the buds are in their early stage of development and the sepals are still turgid when the fruit approaches maturity, whereas in allogamous, post-anthesis fecund flowers the perianth remains in place but begins to dry out a few days after pollination and becomes papyraceous with age. We found that there is no way to distinguish between the fruit of a cleistogamous plant and the developing fruit of a recent pollinated flower, the perianth of which is still turgid, without opening the perianth and looking at the remnant of the rostellum, which is completely absent in cleistogamous forms. This may perhaps explain why the number of documented specimens of ‘gravid’ encyclias is scanty.

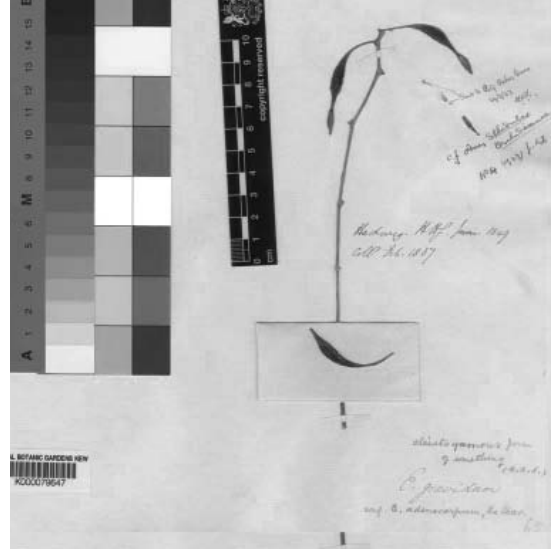


FIGURE 10. The holotype of *Epidendrum gravidum*. Reproduced with permission by the Board of Trustees, Royal Botanic Gardens, Kew.

Before World War II, Blanche Ames (Ames, 1923) illustrated the flower of another Mexican specimen collected by Purpus in Zacualpan, Veracruz, at that time kept in Schlechter’s herbarium in Berlin but later destroyed (Fig. 11, A), which may correspond morphologically to the species originally described by Lindley. A Puerto Rican cleistogamous specimen was documented by Ackerman (1995), who noted that the perianth parts of the opened flower are not exactly zygomorphic, probably because of some constraint during development (Fig. 11, B). It is likely that another collection recorded by Schweinfurth (1940) from Maricao in Puerto Rico (*Kevorkian 6177*, AMES) belongs to this concept, because, according to the collector, the “flowers [...] does not open.”

We have documented two cleistogamous specimens from plants growing in the living collections of Lankester Botanical Garden, University of Costa Rica. We had the plants growing under controlled conditions for a while, and cleistogamy in this case appears to be genetically controlled. Even though the two individuals share some of the morphological features expected for this taxon, they also differ in a number of characters, in particular the shape of the petals, the length of the labellar isthmus, the relative thickening of the veins on the midlobe, and the shape of the midlobe, which

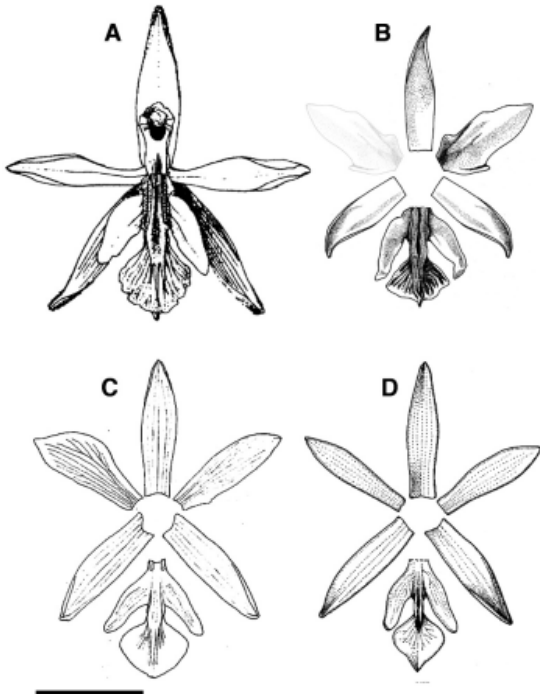


FIGURE 11. Cleistogamous *Encyclia*. A - Mexico, *Purpus* s.n., (B, destroyed); B - Puerto Rico; C - Costa Rica, *Pupulin* 6670 (JBL-Spirit); D - Costa Rica, *Pupulin* 5377 (JBL-Spirit). A, drawn by Blanche Ames, from Ames, 1923; B, drawn by M. Enríquez, from Ackerman 1995; C-D, drawn by F. Pupulin. Scale bar = 1 cm.

is almost rhombic in one specimen and transversely ovate in the other (Fig. 11, C—D). More interesting is the fact that whereas one of the specimens lacks locality data, the other was collected in a region where only one other species of *Encyclia*, *E. ceratistes*, occurs naturally. During field work for the present treatment, we are making extensive collections of *Encyclia* specimens in this region, and to date we have observed only a single cleistogamous specimen. This may perhaps indicate that ‘gravid’ encyclias do not form populations, one of the essential requirements to be considered a good species, and are nothing more than occasional mutants.

When we compare the few documented specimens of cleistogamous *Encyclia*, it is evident that their similarity is superficial (Fig. 11, A-D), even when we could attribute these differences to some degree of deformity of the perianth parts, which do not spread out at the end of development. But which character (or set of characters) should properly define *E. grvida*?

Apart from the vegetative architecture, which is largely uniform in *Encyclia* species, and the features of the floral parts, which in turn vary considerably among individuals, the only shared character we observed in cleistogamous flowers is the absence of a definite rostellum, which would prevent the displacement of the pollen toward the stigmatic cavity. However, due to the paucity of available records of fecund plants of *Encyclia* to compare, this key feature alone is insufficient to assign cleistogamous individuals to a well-characterized species, and the possibility that the records simply represent self-pollinating forms belonging to different taxa cannot be discarded on the basis of floral morphology. The comparison of genetic sequences of individuals tentatively assigned to *E. grvida* with those of other sympatric species of *Encyclia* could represent an important step toward a better circumscription of the taxa in this group and the appreciation of the evolutionary significance of different pollination strategies.

A case for barcoding

In recent years, sequencing of plant genomes has been regarded as a powerful tool to assess the consistency of specific concepts and disclose cryptic taxa difficult to tell apart with traditional taxonomic methods, mainly through species DNA barcoding. However, even if at some stage clearly divergent barcodes can be used as the basis for nomenclatural decisions, barcodes by themselves are insufficient to describe new species. Species descriptions are scientific hypotheses that should be supported by a variety of other, including non-molecular, data sets. One of the necessary steps to place barcoding within the context of a rich taxonomic approach is the analysis of the range of intra- and interspecific variations in morphological characters, helping to prioritize which morphospecies and which individuals in problematic species complexes should be sequenced.

Application of the same species concept in taxonomically difficult groups like *Encyclia*, where hybridization may be frequent and occur over large areas, is still debatable (see Dressler and Pollard, 1976). DNA-based techniques may represent a unique opportunity to improve and simplify our hypotheses about species, but it is advisable they be used together with other disciplines such as phylogeography,

comparative morphology, population genetics, ecology, development, and pollination biology to delimit the units of orchid diversity.

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THE LEAVES GOT IT RIGHT AGAIN: DNA PHYLOGENETICS SUPPORTS A SISTER-GROUP RELATIONSHIP BETWEEN *EURYSTYLES* AND *LANKESTERELLA* (ORCHIDACEAE: SPIRANTHINAE)

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ABSTRACT. In spite of noticeable non-floral similarities such as their unusual epiphytic habit and vegetative morphology, *Eurystyles* and *Lankesterella* have been regarded by taxonomists who rank floral characters above all other sources of information as only distantly related. Here we assess the phylogenetic relationships of these genera, analyzing over 4500 characters of nuclear (nrITS) and plastid (*matK-trnK*, *trnL-trnF*) DNA sequences from 29 species/22 genera of Spiranthinae (plus appropriate outgroups); three structurally distinctive species of *Eurystyles* and two of *Lankesterella* were included. Both our parsimony and Bayesian phylogenetic analyses recovered *Eurystyles* and *Lankesterella* as sister taxa with strong internal support. The *Eurystyles/Lankesterella* clade is in turn supported as sister to the “Spiranthes clade.” Our results agree with previous interpretations of a close relationship between these two genera based on their shared epiphytic habit and similar vegetative morphology, indicating that floral morphology is evolutionarily labile in these groups and thus less reliable as an indicator of phylogenetic relationship than more conservative vegetative morphology.

RESUMEN. A pesar de notables similitudes en características no florales, tales como su desusado hábito epífita y su morfología vegetativa, *Eurystyles* y *Lankesterella* han sido considerados como sólo distantemente relacionados entre sí por los taxónomos que valoran los atributos florales por encima de cualquier otra fuente de información. En este trabajo evaluamos las relaciones filogenéticas de estos géneros analizando más de 4500 caracteres de secuencias de ADN nuclear (nrITS) y de plástidos (*matK-trnK*, *trnL-trnF*) de 29 especies/22 géneros de Spiranthinae (y grupos externos apropiados); tres especies de *Eurystyles* estructuralmente distintas entre sí y dos de *Lankesterella* fueron incluidas. Tanto nuestro análisis de parsimonia como el de inferencia bayesiana recobran a *Eurystyles* y *Lankesterella* como taxones hermanos con fuerte apoyo interno. El clado *Eurystyles/Lankesterella* a su vez está apoyado como hermano del “clado Spiranthes.” Nuestros resultados concuerdan con interpretaciones previas de una relación cercana entre *Eurystyles* y *Lankesterella* basadas en el hábito epífita que comparten y su similar morfología vegetativa, indicando que la morfología floral es evolutivamente lábil en estos grupos y por lo tanto menos confiable como indicador de relaciones filogenéticas que la morfología vegetativa, más conservadora.

KEY WORDS: Orchidaceae, Spiranthinae, *Eurystyles*, *Lankesterella*, molecular phylogeny

“It is ironic that within a family known for its spectacular flowers and specialized floral morphology, vegetative characters are quickly gaining recognition as a better indicator of phylogenetic relationships for particular groups.”

Kenneth M. Cameron, *American Journal of Botany* 92: 1025-1032 (2005).

The genera *Eurystyles* Wawra and *Lankesterella* Ames share several attributes that make them unique

in subtribe Spiranthinae. The most obvious of these is their consistently epiphytic habit (Fig. 1A-B, 2A-B), in contrast with the ubiquitous terrestrial habit displayed by the nearly 40 other genera currently recognized in the subtribe (Schlechter, 1920; Dressler, 1981, 1993; Balogh, 1982; Garay, 1982; Burns-Balogh *et al.*, 1985; Szlachetko, 1992; Salazar, 2003, 2005; Salazar *et al.*, 2003). Furthermore, plants in both of these genera consist of small rosettes of lustrous, usually ciliate

leaves that persist during several growth seasons, *i.e.* they are not deciduous as in other Spiranthinae, and their roots are fasciculate but comparatively slender instead of tuberous. Indeed, *Eurystyles* and *Lankesterella* are vegetatively so similar to one another that, in the absence of inflorescences, it is not always easy to identify to which genus a particular plant belongs (Johnson, 2001; Soto, 1993).

In contrast to their vegetative similarity, these two genera show striking differences in their reproductive structures. On the one hand, in *Eurystyles* the raceme is condensed (thus appearing capitate) and the numerous flowers are densely arranged in a spiral (Fig. 1B-D), whereas in *Lankesterella* the inflorescence consists of a lax, one-sided raceme bearing a few (usually 1-4) flowers (Fig. 2B, C). On the other hand, flowers of the two genera also differ in various structural details. In *Eurystyles* the bases of the sepals are inflated but do not form a distinct, retrorse spur. The base of the labellum usually is clawed and bears a retrorse, fleshy lobule at each side above the claw (except in *Eurystyles* subgenus *Pseudoëurystyles* (Hoehne) Szlach., in which the lip is sessile and lacks basal lobules). The gynostemium is free and elongated, and the rostellum, when present, usually forms a shallowly notched, membranaceous rostellum remnant upon removal of the pollinarium (Fig. 1E-H). In contrast, flowers of *Lankesterella* bear a retrorse spur, sessile labellum with marginal thickenings near the base, abbreviated gynostemium, and hard, pointed rostellum remnant (Fig. 2C-F).

The noticeable similarity in habit and vegetative structure between *Eurystyles* and *Lankesterella* led Dressler (1981), Soto (1993), and Salazar (2003, 2005) to advocate a close relationship between these two genera. Nevertheless, taxonomists who have preferred to classify orchids on the basis of floral characters to the exclusion of virtually all other biological evidence have disregarded such non-floral resemblance and instead considered these genera as distantly related, placing them in different generic alliances (Schlechter, 1920; Balogh, 1982; Burns-Balogh *et al.*, 1985) or even distinct subtribes (Szlachetko, 1995; Szlachetko and Rutkowski, 2000; Szlachetko *et al.*, 2005).

A recently published molecular phylogenetic analysis of Spiranthinae based on sequences of nuclear ribosomal ITS DNA (nrITS; Górniak *et al.*, 2006)

sustained a sistergroup relationship between *Eurystyles* and *Lankesterella*. However, that analysis included only one species each of *Eurystyles* and *Lankesterella* and was based on a single DNA region, which raised the question of whether such an “unorthodox” result (by floral taxonomist’s standards) would hold true if increased numbers of taxa and characters were included in the analysis. In this study, we assess the phylogenetic position of *Eurystyles* and *Lankesterella* by conducting cladistic analyses with expanded sampling of both taxa and characters and also by analyzing nuclear (nrITS) as well as plastid (*matK-trnK* and *trnLtrnF*) DNA sequence data. Our aims are to clarify the relationships of *Eurystyles* and *Lankesterella* with one another and other members of Spiranthinae, as well as evaluate the reliability of vegetative versus floral characters as predictors of phylogenetic relationships in these genera against the background of the DNA sequence trees.

Materials and methods

Taxonomic sampling—Exemplars of three species of *Eurystyles*, two of *Lankesterella*, and 24 additional species of Spiranthinae (comprising in total 22 genera) were analyzed in this study. Representative species of all other subtribes of Cranichideae *sensu* Salazar *et al.* (2003, 2009) were used as outgroups. A list of the species analyzed with voucher information and GenBank accessions for DNA sequences is given in Table 1.

Although we sampled only three of the about 20 species of *Eurystyles*, they represent much of the reproductive structural variation displayed by the genus, which is reflected in the fact that the three species have been assigned by taxonomists to different sections, subgenera, and even different genera in the case of “*Synanthes*” [= *Eurystyles*] *borealis* (Heller) Burns-Bal., Robinson & Foster (Burns-Balogh *et al.*, 1985; Szlachetko, 1992). As for *Lankesterella*, the genus encompasses six to eight species (Garay, 1982; Szlachetko *et al.*, 2005), all of which seem to be relatively uniform in most vegetative and reproductive morphological attributes.

Molecular methods—DNA extraction, gene amplification, and sequencing were carried out using standard protocols explained in Salazar *et al.* (2003) and Figueroa *et al.* (2008). Bidirectional sequence reads were obtained for all the DNA regions; chromatograms

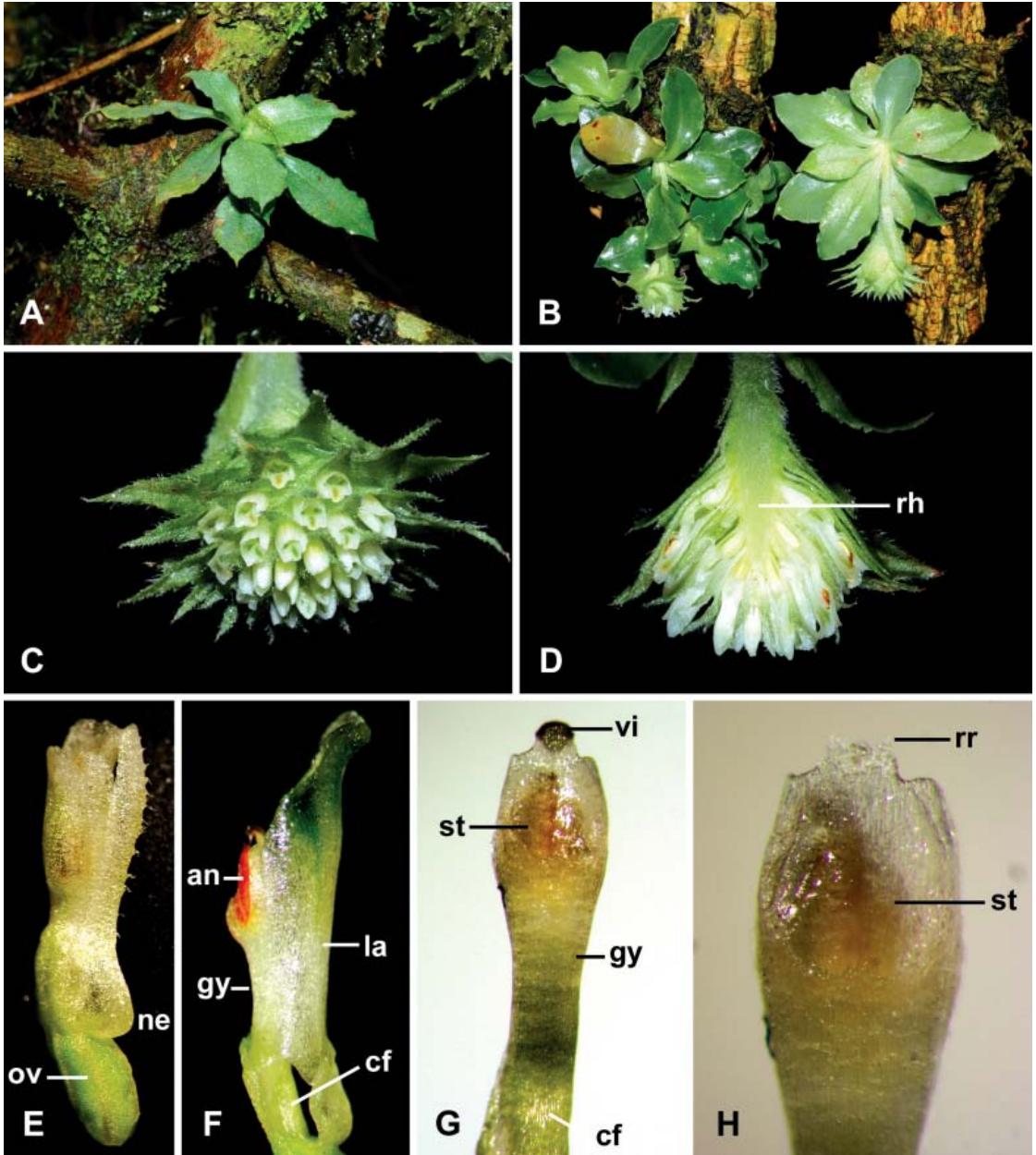


FIGURE 1. Morphology of *Eurystyles*. A. Plant of *Eurystyles cotyledon* in situ in a cloud forest of southern Ecuador. B. Flowering plants of *E. auriculata* (left) and *E. cotyledon* (right) in cultivation. C. Inflorescence of *E. cotyledon* from below. D. Longitudinal section of the same inflorescence. E. Individual flower of *E. cotyledon* viewed obliquely from above and the side. F. Labellum and gynostemium of previous flower after the sepals and petals were excised. G. Gynostemium of *E. cotyledon* from below. H. Apex of previous gynostemium after removal of the pollinarium, showing the broadly notched rostellum remnant. Abbreviations: an = anther; cf = column foot; gy = gynostemium; la = labellum; ne = nectary; ov = ovary; rh = rachis of the raceme; rr = rostellum remnant; st = stigma; vi = viscidium. (B left, from Salazar 7646; B right-H, from Salazar 7642). Photographs by G. A. Salazar.

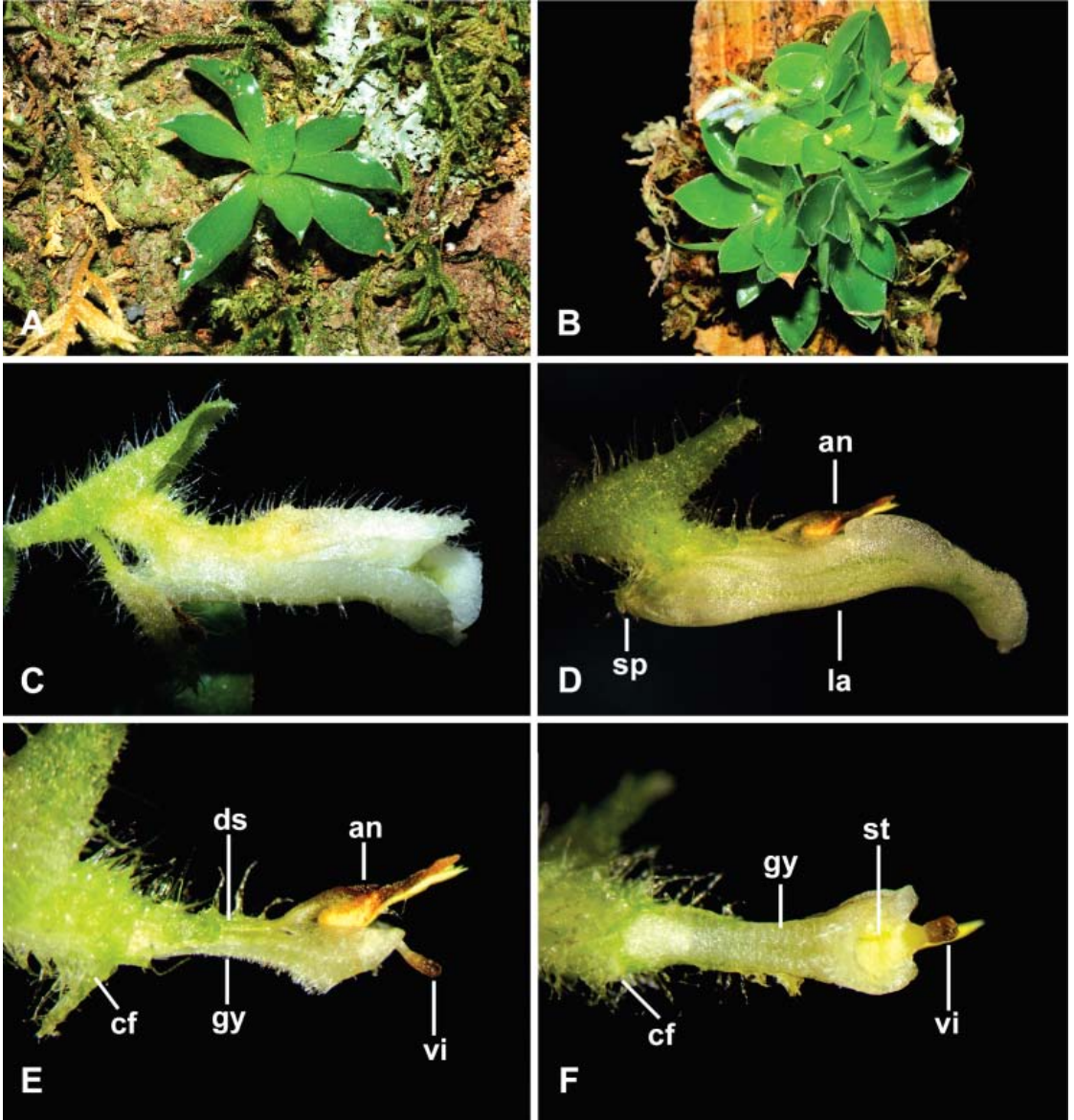


FIGURE 2. Morphology of *Lankesterella*. A. *Lankesterella gnoma* in situ in a mata atlántica of southeastern Brazil. B. Flowering plant of *Lankesterella ceracifolia* in cultivation. C. Close-up of a single-flowered inflorescence of the same plant. D. Labellum and column of previous flower after the sepals and petals were excised. E. Gynostemium from the side. F. Gynostemium from below. Abbreviations: an = anther; cf = column foot; ds = base of dorsal sepal (or ovary apex?) adnate to gynostemium; gy = gynostemium; la = labellum; sp = spur; st = stigma; vi = viscidium. (B-F from Salazar 7535). Photographs by G. A. Salazar.

were edited and assembled with Sequencher version 4.8 (GeneCodes Corp.). Alignment of the sequences was achieved by visual inspection in order to maximize sequence similarity (Simmons, 2004). No data were excluded from the analyses due to unambiguous

alignment or for other reason, but individual gap positions were treated as missing data.

Cladistic analyses—From our previous molecular phylogenetic studies of Spiranthinae and other

TABLE 1. Taxa studied, voucher information, and GenBank accessions.

Taxon	Voucher	Gen Bank Accession		
		<i>trnL-F</i>	<i>matK-trnK</i>	ITS
Subtribe Achlydosinae M.A.Clem. & D.L.Jones				
<i>Achlydosa glandulosa</i> (Schltr.) M.A.Clem. & D.L.Jones	New Caledonia, <i>Clements D-285</i> , CANB	AJ544506	AJ543950	AJ539525
Subtribe Chloracinae Rchb.f.				
<i>Chloraea magellanica</i> Hook.f.	Chile, <i>Ryan 1</i> , K (spirit)	AJ544504	AJ543948	AJ539523
<i>Gavilea lutea</i> (Pers.) M.N.Correa	Chile, <i>Ryan 3</i> , K (spirit)	AJ544505	AJ543949	AJ539524
Subtribe Cranichidinae Lindl.				
<i>Aa colombiana</i> Schltr.	Colombia, <i>Aldana 2</i> , ANDES	AM412731	AM900802	AM419766
<i>Altensteinia fimbriata</i> Kunth	Ecuador, <i>Salazar 6789</i> , MEXU (spirit)	AM412737	AM900801	AM419765
<i>Cranichis engelii</i> Rchb.f.	Ecuador, <i>Schott s.n.</i> , K (spirit)	AM412721	AM900814	AM419779
<i>Galeoglossum thysanochilum</i> (B.L.Rob. & Greenm.) Salazar	Mexico, <i>Tenorio 17900</i> , MEXU	AM412725	AM900810	AM419775
<i>Gomphichis caucana</i> Schltr.	Colombia, <i>Díaz 159</i> , ANDES	AM412736	AM900805	AM419770
<i>Ponthieva racemosa</i> (Walt.) C.Mohr	Mexico, <i>Salazar 6049</i> , MEXU	AJ544490	AJ543936	AJ539508
<i>Porphyrostachys pilifera</i> Rchb.f.	Peru, <i>Whalley s.n.</i> , K (photograph)	AJ544496	AJ543942	AJ539514
<i>Prescottia plantaginea</i> Lindl.	Brazil, <i>Salazar 6350</i> , K (spirit)	AJ544493	AJ543939	AJ539511
<i>Pterichis habenarioides</i> Schltr.	Colombia, <i>Aldana 12</i> , COL	AJ544491	AJ543937	AJ539509
<i>Stenoptera ecuadorana</i> Dodson & C.Vargas	Ecuador, <i>Salazar 6357</i> , K (spirit)	AJ544494	AJ543940	AJ539512
Subtribe Galeottiellinae Salazar & M.W.Chase				
<i>Galeottiella sarcoglossa</i> (A.Rich. & Galeotti) Schltr.	Mexico, <i>Jiménez 2334</i> , AMO	AJ544500	AJ543945	AJ539518
Subtribe Goodyerinae Klotzsch				
<i>Ludisia discolor</i> (Ker-Gawl.) A.Rich.	Tropical Asia (cultivated specimen), <i>Salazar 6354</i> , K (spirit)	AJ544466	AJ543911	AJ539483
<i>Pachyplectron arifolium</i> Schltr.	New Caledonia, <i>Chase 529</i> , K	AJ544503	AJ310051	AJ539522
Subtribe Manniellinae Schltr.				
<i>Manniella cypripedoides</i> Salazar, T.Franke, Zapfack & Benkeen	Cameroon, <i>Salazar et al. 6323</i> , YA	AJ544498	AJ543943	AJ539516

Taxon	Voucher	Gen Bank Accession		
		<i>trnL-F</i>	<i>matK-trnK</i>	ITS
Subtribe Pterostylidinae Pfitz.				
<i>Pterostylis curta</i> R.Br.	Australia, <i>Chase 572</i> , K	AJ544507	AJ543951	AJ539526
Subtribe Spiranthinae Lindl.				
<i>Aulosepalum tenuiflorum</i> (Greenm.) Garay	Mexico, <i>Salazar 6017</i> , MEXU	–	AJ543919	–
<i>Aulosepalum tenuiflorum</i> (Greenm.) Garay	Mexico, <i>Salazar et al. 6150</i> , MEXU	AJ544474	–	AJ539591
<i>Beloglottis costaricensis</i> (Rchb.f.) Schltr.	Mexico, <i>Soto 8129</i> , MEXU	AJ544475	AJ543920	AJ539492
<i>Coccineorchis cernua</i> (Lindl.) Garay	Panama, <i>Salazar et al. 6249</i> , MEXU (spirit)	AJ544485	AJ543930	AJ539502
<i>Cyclopogon epiphyticum</i> (Dodson) Dodson	Ecuador, <i>Salazar 6355</i> , K	AJ544482	AJ543927	AJ539499
<i>Deiregyne diaphana</i> (Lindl.) Garay	Mexico, <i>Salazar et al. 6172</i> , MEXU	AJ544467	AJ543912	AJ539484
<i>Dichromanthus aurantiacus</i> (La Llave & Lex.) Salazar & Soto Arenas	Mexico, <i>Salazar 6351</i> , K (spirit)	AJ544468	AJ543913	AJ539485
<i>Dichromanthus cinnabarinus</i> (La Llave & Lex.) Garay	Mexico, <i>Linares 4469</i> , MEXU	AJ544469	AJ543914	AJ539486
<i>Eltroplectris calcarata</i> (Sw.) Garay & H.R.Sweet	Brazil, <i>Soares s.n.</i> , K (photograph)	AJ519452	AJ519450	AJ519448
<i>Eurystyles auriculata</i> Schltr.	Costa Rica, <i>Dressler s.n.</i> , MEXU (spirit)	FN556166	FN556171	FN556161
<i>Eurystyles borealis</i> A.H.Heller	Mexico, <i>Soto 9149</i> , AMO	AJ544480	AJ543925	AJ539497
<i>Eurystyles cornu-bovis</i> Szlach.	Panama, <i>Maduro 230</i> , FLAS	FN556167	FN556172	FN556162
<i>Funkiella hyemalis</i> (A.Rich. & Galeotti) Schltr.	Mexico, <i>Salazar et al. 6128</i> , MEXU	AJ544478	AJ543923	AJ539495
<i>Hapalorchis lineatus</i> (Lindl.) Schltr.	Puerto Rico, <i>Ackerman 3648</i> , UPRRP	FN556170	FN556175	FN556165
<i>Lankesterella ceracifolia</i> (Barb.Rodr.) Ames	Argentina, <i>Salazar 7535</i> , MEXU (spirit)	FN556169	FN556174	FN556164
<i>Lankesterella gnoma</i> (Kraenzl.) Hoehne	Brazil, <i>Warren s.n.</i> , K (spirit)	FN556168	FN556173	FN556163
<i>Mesadenella petenensis</i> (Standl. & L.O.Williams) Garay	Mexico, <i>Salazar 6069</i> , MEXU	AJ544486	AJ543931	AJ539503
<i>Mesadenus lucayanus</i> (Britt.) Schltr.	Mexico, <i>Salazar 6043</i> , MEXU	AJ544471	AJ543916	AJ539488

Taxon	Voucher	Gen Bank Accession		
		<i>trnL-F</i>	<i>matK-trnK</i>	ITS
Subtribe Spiranthinae Lindl.				
<i>Microthelys minutiflora</i> (A.Rich. & Galeotti) Garay	Mexico, Salazar <i>et al.</i> 6129, MEXU	AJ544477	AJ543922	AJ539494
<i>Odontorrhynchus variabilis</i> Garay	Chile, Wallace 130/85, CANB	AJ544481	AJ543926	AJ539498
<i>Pelexia adnata</i> (Sw.) Poit. ex Spreng.	Mexico, Salazar 6012, MEXU	AJ544484	AJ543929	AJ539501
<i>Sacoila lanceolata</i> (Aubl.) Garay	Brazil, Da Silva 874, MG	AJ544529	AJ543933	–
<i>Sacoila lanceolata</i> (Aubl.) Garay	Panama, Förther 2545, M	–	–	AJ539504
<i>Sarcoglottis acaulis</i> (J.E.Sm.) Schltr.	Trinidad, Salazar 6356, K (spirit)	AJ544483	AJ543928	AJ539500
<i>Schiedeella faucisanguinea</i> (Dod) Burns-Bal.	Mexico, Jiménez <i>s.n.</i> , AMO	AJ544479	AJ543924	AJ539496
<i>Schiedeella llaveana</i> (Lindl.) Schltr.	Mexico, Salazar 6073, MEXU	AJ544470	–	–
<i>Schiedeella llaveana</i> (Lindl.) Schltr.	Mexico, Salazar 6105, MEXU	–	AJ543915	AJ539487
<i>Spiranthes cernua</i> (L.) Rich.	USA, Nickrent 4188, MEXU	AJ544472	AJ543916	AJ539489
<i>Spiranthes spiralis</i> (L.) Cheval.	United Kingdom, Bateman <i>s.n.</i> , K (spirit)	AJ544473	AJ543918	AJ539490
<i>Stenorrhynchus glicensteinii</i> Christenson	Mexico, Salazar 6090, MEXU	AJ544487	AJ543532	AJ539505
<i>Svenkoeltzia congestiflora</i> (L.O.Williams) Burns-Bal.	Mexico, Salazar 6143, MEXU	AJ544476	AJ543921	AJ539493

Cranichideae (Salazar *et al.*, 2003, 2009; Figueroa *et al.*, 2008), it has become evident that combined analyses of the DNA sequence data that we are analyzing here increases resolution and internal clade support as compared with the separate analyses. Therefore, our approach here was to analyze all data sets in combination. The combined matrix was thus analyzed by two different phylogenetic methods: maximum parsimony and Bayesian inference. The parsimony analysis was conducted with the computer program PAUP* version 4.02b for Macintosh (Swofford, 2002), and consisted of a heuristic search with 1000 replicates of random taxon addition for the starting trees and tree rearrangements using tree bisection-reconnection (“TBR”) branch-swapping; the option “MULTREES” was activated (to allow for storage in memory of multiple trees), and all most-parsimonious trees (MPTs) were saved. All

characters were treated as unordered and had equal weights (Fitch, 1971). Internal support for clades was assessed by bootstrapping (Felsenstein, 1985), for which 300 bootstrap replicates were performed, each with 20 replicates with random taxon addition and TBR branch-swapping, keeping up to 20 most-parsimonious trees from each addition replicate. The Bayesian analysis was carried out using the program MrBayes version 3.1.2 for Macintosh (Ronquist *et al.*, 2005). The best-fitting models of nucleotide substitution for five character partitions (nrITS region, *matK* pseudogene, *trnK* intron, *trnL* intron, and *trnL-trnF* intergenic spacer) were selected using the Akaike Information Criterion (Akaike, 1974) with the program Modeltest 3.7 (Posada and Crandall, 1998). In all instances, a six-parameter model with among-site rate heterogeneity modeled according to a gamma distribution and a proportion of invariant

characters was selected, except that for the *trnK* intron there were no invariant characters. Thus, two character partitions were declared in MrBayes, one including the *trnK* intron and another encompassing all the other sequence data, specifying the appropriate models. All model parameters were unlinked among the five character partitions, allowing each group of characters to have its own set of parameters (Ronquist *et al.*, 2005). Two simultaneous analyses were run for 1,000,000 generations, sampling from the trees every hundredth generation under the default conditions of MrBayes for the Markov chains. The first 250,000 generations (2500 trees) of each run were discarded as the burn-in. Inferences about relationships and posterior probabilities of clades (PP) were based on a majority-rule summary tree constructed by pooling the remaining 15,000 trees.

Results

Parsimony analysis — The concatenated data set consisted of 4549 characters, 1066 (23%) of which were potentially parsimony-informative. The heuristic search recovered six most parsimonious trees with a length of 4381 steps, consistency index (excluding uninformative characters) of 0.45, and retention index of 0.64. The six cladograms differed only in the resolution among species of *Eurystyles* and in whether or not the *Stenorrhynchos* and *Pelexia* clades (see below) are sisters with one another. However, none of the alternative resolutions received bootstrap support [BS] greater than 50%. One of the six trees is shown in Figure 3A. The same tree, which is topologically identical to the Bayesian consensus and on which bootstrap percentages and posterior probabilities (from the Bayesian analysis; see below) were included for the pertinent clades, is depicted in Figure 3B. Four major clades were recovered within strongly supported Spiranthinae (BP 100; Fig. 3A, B): 1) the *Stenorrhynchos* clade (*Stenorrhynchos glicensteinii* through *Sacoila lanceolata*; BP 98%); 2) the *Pelexia* clade (*Coccineorchis cernua* through *Pelexia adnata*; BP 80%); 3) a strongly supported clade consisting of *Eurystyles* and *Lankesterella* (BP 100%); and 4) a strongly supported *Spiranthes* clade (*Hapalorchis lineatus* through *Dichromanthus cinnabarinus*; BP 86%). *Eurystyles* and *Lankesterella* are in turn

moderately supported as collective sisters to the *Spiranthes* clade.

Bayesian analysis — The majority-rule consensus calculated from 15,000 trees from the Bayesian analysis was fully resolved and topologically identical to the parsimony tree of Figure 3A. Posterior probabilities (PP) of clades are displayed in Figure 3B. Like parsimony, the Bayesian analysis recovered a strongly supported clade consisting of *Eurystyles* and *Lankesterella* (PP 1.00).

Discussion

Our parsimony and Bayesian analyses recovered the same phylogenetic patterns, both supporting *Eurystyles* and *Lankesterella* as forming a strongly supported clade. These congruent results are significant, given the disproportionately long branches subtending *Eurystyles* and *Lankesterella* (in the parsimony tree portrayed Figure 3A, the branch leading to *Eurystyles* is 124 steps long, whereas that of *Lankesterella* is 116 steps long). It has been proposed that parsimony may be inconsistent as a method of phylogeny reconstruction when different groups within a lineage evolve at dissimilar rates (Felsenstein, 1978, 2004; Hendy & Penny, 1989) because of an analytical artefact known as “long-branch attraction,” which occurs when long-branched lineages that are not each other’s closest relatives are grouped because of similarities due to independent substitutions to the same base from different ancestral bases (e.g. Lewis, 1998; Sanderson *et al.*, 2000; Anderson & Swofford, 2004; Bergsten, 2005). Methods based on stochastic models of nucleotide substitution such as maximum likelihood and Bayesian inference, however, have been shown to be less sensitive to such conditions (see Swofford *et al.*, 1996; Lewis, 1998; Felsenstein, 2004; Bergsten, 2005). In these analyses, both parsimony and Bayesian inference recovered the same tree topology, indicating that the strongly supported placement of *Eurystyles* and *Lankesterella* as sister groups is not an artefact of long branches misleading parsimony.

Our results are in full agreement with the remarkable similarities in habit and vegetative structure between *Eurystyles* and *Lankesterella*, in spite of their disparity in reproductive attributes. Such disparity might actually have been overstated, and

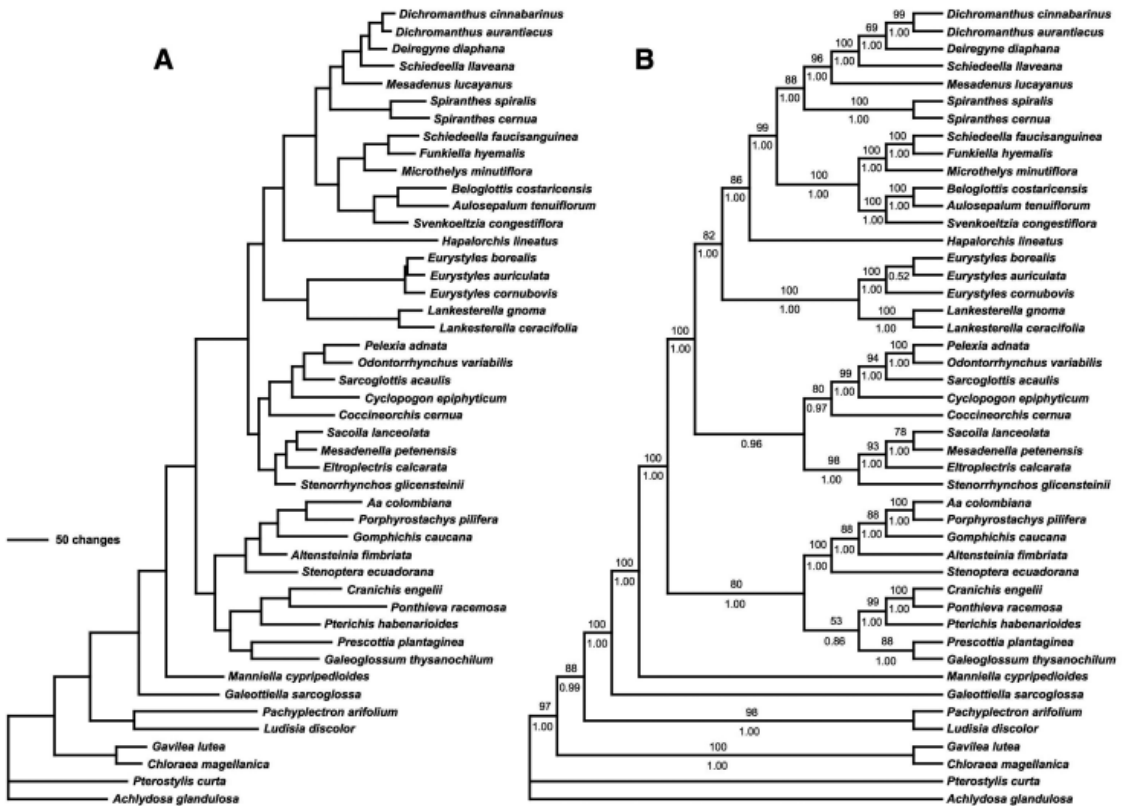


FIGURE 3. Phylogenetic relationships of *Eurystyles*, *Lankesterella*, and other Spirantheae inferred from combined nuclear ITS and plastid *trnK-matK* and *trnLtrnF* DNA sequences. A. One of the six shortest trees recovered by the parsimony analysis, with branch lengths drawn proportional to the number of character changes supporting them; arrows point to clades that collapse in the strict consensus. B. Same parsimony tree as in A, which was topologically identical to the Bayesian summary tree, and on which bootstrap percentages (numbers above branches) and posterior probabilities from the Bayesian analysis (numbers below branches) are superimposed (see text).

upon closer scrutiny the morphological gap between these genera appears not as large as it would appear at first glance. For instance, and quoting Szlachetko (1992), labellum structure between *Eurystyles* subgen. *Pseudoëurystyles* and *Lankesterella* “is almost identical.” Overall flower structure is much the same in both genera, and although species of *Eurystyles* lack the pointed retrorse spur, the base of their floral tube always is distinctly inflated (compare Fig. 1E and 2D).

Gynostemium morphology between these genera as described in the literature seems to be dissimilar, with the gynostemium of *Lankesterella* often being interpreted as relatively short, with an “obscure to short” column part (Szlachetko & Rutkowski, 2000; Szlachetko *et al.*, 2005) and a prominent column

foot. However, in the fresh material of *Eurystyles* and *Lankesterella* that we have examined the gynostemium look similar, with the main difference being that, in *Lankesterella*, the basal portion of the dorsal sepal or the apex of the ovary (or both) is adnate to the back of the gynostemium (indicated in Figure 2E as “ds”). Were it not for this adnation, the proportions of column part to column foot of both genera would look even more similar.

The most distinctive feature separating the two genera is the rostellum, which after removal of the pollinarium in *Lankesterella* usually leaves a hard narrow point that is absent in *Eurystyles*. However, *Eurystyles* shows substantial structural variation (see Szlachetko, 1992; Szlachetko & Rutkowski, 2000),

and both *Eurystyles* and *Lankesterella* are known for their frequent, seemingly autogamous forms, in which rostellum and viscidia are reduced, non-functional or missing altogether (Szlachetko, 1992; Salazar, 2003). These phenomena indicate that rostellum morphology in these groups is evolutionarily labile.

The abovementioned differences in reproductive structures between these two genera are almost certainly related to different pollination mechanisms. Unfortunately, nothing is known about their natural pollination besides the aforementioned recurrence of autogamy. The inflorescences of *Eurystyles auriculata* and *E. cotyledon* produce conspicuous, pleasant, diurnal odors, which might play a role in pollinator attraction. Their pendulous, dense clusters of tubular, fragrant flowers with nectar at the bottom of the floral tube may be pollinated by small insects (possibly lepidopterans) that probe many flowers in search of food. However, we have had limited opportunities to examine fresh inflorescences of *Lankesterella* in the field, and we are unable at this time to confirm whether they are also fragrant or not.

Given the unusual (in subtribe Spiranthinae) epiphytic habit of *Eurystyles* and *Lankesterella* on the one hand and their long branches in the molecular tree on the other, one has to ask whether there may be a relationship between epiphytism and an accelerated rate of molecular evolution in these genera. However, the branch of *Hapalorchis lineata* (Fig. 3A), a terrestrial species, is even longer (194 steps). Therefore, drawing conclusions in this respect is not possible at this time, but it will be interesting to address this matter as more sequences of these and other genera of the subtribe become available.

In closing, it is worth mentioning that our results mirror those of Cameron's (2005) molecular phylogenetic study of Malaxideae (from which our epigraph was taken) in showing that at least in some particular orchid groups vegetative structure might be a better predictor of phylogenetic relationships than floral attributes. In both the epidendroid tribe Malaxideae and the orchidoid subtribe Spiranthinae, vegetative features prove to be more conservative than floral details. Indeed, speciation in these groups often appears to involve the evolution of different floral features in closely allied species, with the result that the species are pollinated by different agents,

or in different ways, and the species coexist without interbreeding. There may also be parallelisms in floral features between quite different groups, as in *Trichocentrum* Poepp. & Endl. and other Oncidiinae, flowers of which mimic the flowers of Malpighiaceae, with or without oil glands (see Chase *et al.*, 2009 and references included there). Thus, one might expect that floral features are more variable and vegetative features more conservative, despite unwarranted assumptions to the contrary (e.g. Szlachetko & Rutkowski, 2000). This, of course, is a generalization. There are certainly parallelisms to be found among vegetative features just as there are among floral features. When in doubt, trust neither the leaves nor the flowers, but the DNA.

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ORCHID SEED STORES FOR SUSTAINABLE USE: A MODEL FOR FUTURE SEED-BANKING ACTIVITIES

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ABSTRACT. Orchid Seed Stores for Sustainable Use (OSSSU) is a three-year UK Darwin Initiative project with the primary objective of setting up a global network of orchid seed banks, focusing initially on orchid biodiversity hotspots in Asia and Latin America. At the time of writing there are 31 participating institutions in 22 countries. In the longer term, our aim is to expand the network to include more institutions from around the world, from African countries in particular. We are confident that good-quality dry orchid seed has the potential to survive for many decades at conventional seed bank temperatures of around -20 °C. Participating institutions have been provided with seed storage tubes, together with funding to purchase a dedicated chest freezer and some consumables. Common protocols have been agreed to allow, for the first time, comparison of germination of seed of more than 250 orchid species from tropical and temperate countries over a wide range of habitat types on one germination medium (Knudson C) and to compare long-term viability. The role of OSSSU in both *ex situ* and *in situ* conservation is illustrated by the case of *Cattleya quadricolor*, a Colombian endemic. The story of *C. quadricolor* is a familiar one: loss of habitat combined with illegal collection of this beautiful species by commercial interests. However, here is a project in which all of the pieces of the orchid conservation jigsaw puzzle fit neatly into place: an up-to-date Red List for the orchids of Colombia to assist with targeting rare and endangered species, a National Action Plan for *Cattleya* species, participation in OSSSU, a group of amateur and professional growers with the necessary expertise to germinate seed for the project and produce seedlings, a committed commercial grower willing to provide plants for pollination and seed harvest, and a botanical garden willing to act as a focal point and to re-introduce plants of *C. quadricolor* raised by the project into a number of secure sites.

RESUMEN. Los Almacenes de Semillas de Orquídeas para Uso Sostenible (Orchid Seed Stores for Sustainable Use – OSSSU por sus siglas en inglés) es una iniciativa de tres años del proyecto Iniciativa Darwin del Reino Unido, con el principal objetivo de establecer una red global de bancos de semillas de orquídeas, enfocándose inicialmente en aquellos “puntos calientes” de biodiversidad en Asia y América Latina. Al momento de escribir este documento, hay 20 instituciones participantes en 16 países. A más largo plazo, nuestro objetivo es el de incluir un mayor número de instituciones de todo el mundo, y especialmente de países africanos. Tenemos la confianza de que una semilla seca de orquídea de buena calidad tiene el potencial sobrevivir durante muchas décadas bajo condiciones de temperatura de un banco de semillas convencional de alrededor de -20 °C. Las instituciones participantes han recibido tubos para el almacenamiento de semillas, junto con los fondos para adquirir una congeladora de baúl específicamente dedicada así como algunos de los suministros consumibles necesarios. Se ha acordado una serie de protocolos, que por primera vez permitirán la comparación de la germinación de semillas de más de 250 especies de orquídeas de países tropicales y templados que cubren una amplia gama de tipos de hábitat en un solo medio de germinación (Knudson C) y compararlas con su viabilidad de largo plazo. El rol de la OSSSU tanto en conservación *ex situ* e *in situ* queda ilustrado con el caso de *Cattleya quadricolor*, una endémica colombiana. La historia de *C. quadricolor* es algo muy familiar: pérdida de hábitat combinada con la ilegal recolección de esta especie tan hermosa debido a intereses comerciales. Sin embargo, aquí tenemos un proyecto en el cual todas las piezas del rompecabezas de la conservación de orquídeas calzan en forma exacta: una Lista Roja actualizada para las orquídeas de Colombia que sería de gran ayuda para apuntar a las especies raras y amenazadas, un Plan Nacional de Acción para las especies de *Cattleya*, la participación en OSSSU, un grupo de cultivadores amateur y profesionales con los conocimientos

necesarios para germinar las semillas para el proyecto y estar en capacidad de producir plántulas, un cultivador comercial comprometido que esté dispuesto a proporcionar plantas para polinización y realizar la cosecha de semillas, y un jardín botánico que está dispuesto a actuar como punto focal para re-introducir plantas de *C. quadricolor* cultivadas por el proyecto a un número de sitios que ofrezcan seguridad.

KEY WORDS: orchids, seed stores, *Cattleya quadricolor*, conservation

The time has arrived to begin banking orchid seeds as a key component of an effective *ex situ* conservation strategy. As long ago as 1984 at the Miami World Orchid Conference it was agreed that orchid seed banking had the potential to make an invaluable contribution to orchid conservation (Anonymous, 1985). Twenty-five years later, as it is becoming increasingly apparent that *in situ* techniques cannot by themselves be sufficient to save all of the world's orchids, *ex situ* techniques are once again recognized as being an essential component of any future integrated conservation strategies (Swarts & Dixon, 2009). Seed banking has been recognized as being the most efficient way of storing large numbers of living plants in one place (FAO, 1996; Linington & Pritchard, 2001).

Adopted in 2002 by the Conference of the Parties of the Convention on Biological Diversity (CBD), the long-term goal of the Global Strategy for Plant Conservation (GSPC) was (and remains) to halt the current and continuing loss of plant biodiversity through the setting of 16 outcome-oriented targets for 2010. The most significant from an *ex situ* perspective is target 8: to have 60% of threatened plant species in accessible *ex situ* collections, preferably in the country of origin, and 10% of them included in recovery and restoration programs (<http://www.bgci.org/plants2010/t8/>). As 2010 approaches, the question we should be asking is: "How far has the orchid community progressed in terms of meeting the above targets?" Indeed, one could also ask if the target was realistic and attainable within the given time frame.

Orchid Seed Stores for Sustainable Use (OSSSU), a Darwin Initiative funded by Defra (the UK government's Department for Environment, Food and Rural Affairs) was originally conceived by Hugh Pritchard and Phil Seaton as a project with the modest aim of storing seeds representing 250 orchid species over a period of three years. Assuming that there are approximately 25,000 orchid species (Dressler, 2005), this would represent around 1% of the world's orchid

flora. At the time of writing this target has already been exceeded by a considerable margin.

Through the funding of collaborative projects that draw on UK biodiversity expertise, Darwin Initiative projects are awarded with the aim of assisting countries that are rich in biodiversity to meet their objectives under one or more of the major biodiversity Conventions (in the case of OSSSU, the Convention on Biological Diversity – the CBD). Accordingly, OSSSU initially focused on orchid-rich countries in Asia and Latin America. At the beginning of the project, participants from China, India, Indonesia, the Philippines, Singapore, Thailand, and Vietnam were invited to a workshop in Chengdu, China. Participants from Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, and Guatemala attended a workshop at Quito Botanical Gardens, Ecuador (Seaton & Pritchard, 2008). Although the world's orchid biodiversity hotspots are mainly concentrated in the tropics (Cribb & Govaerts, 2005), there are also important orchid biodiversity hotspots in cooler regions, explaining the inclusion of the temperate orchid floras of China and Chile.

The workshops provided the opportunity to exchange expertise and ideas and develop common protocols. Participants reflected the wide range of scientific backgrounds and experience found within the membership of the orchid community – from orchid biotechnologists to field biologists -- each contributing his or her own experience and expertise to the project. Once a formal agreement had been signed (a Memorandum of Understanding) with a participating country, the Darwin Initiative provided modest funding to enable each participating country to purchase a small chest freezer dedicated to storing orchid seed at -20 C, plus some consumables. Participants were also supplied with suitable seed-storage vessels.

Despite an early report that some orchid seed retained some viability after storage for up to 20 years under less than ideal conditions (Knudson, 1954), at the time of the 1984 World Orchid Conference there

remained a lack of numerical data for initial germination percentage enabling comparisons to be made with later samplings of stored seed. Although doubts continue to be expressed concerning the potential longevity of orchid seed in dry storage (Neto & Custodio, 2005), part of the problem may lie in the definition of the terms 'long-lived' and 'short-lived'. Certainly a number of studies have shown that, as long as the seed is dried correctly, good-quality seed of many orchid species are likely to remain viable for many decades when stored at -20 C (Seaton & Pritchard, 2003). Nevertheless, the number of species tested has so far been relatively limited. One of the many strengths of OSSSU is that germination data will become available for more than 250 species across many genera in many countries and many different habitats around the globe. All seed accessions will be tested on one medium, Knudson C (Knudson, 1946), to allow a direct comparison to be made between the responses of species and genera. This will be the first time that such information will become available for such a wide range of material. In addition, a sample of each seed lot will be sown on a second medium as a comparison. We already know that Knudson C is not the most suitable medium for all species. Some species may, for example, have a higher percentage germination on Murashige and Skoog medium (Murashige & Skoog, 1962) than on Knudson C. Other species will indeed have a higher percentage germination on Knudson C. Some species will perform better on other media (Arditti *et al.*, 1982), and participants have the opportunity to select media that, according to their own experience, give the optimum germination percentage for each individual species.

One aim of the project is to publish all of the data at the end of the project period and make it available to the wider orchid community. Each participating institute is committed to promoting the project through a public awareness program, and many partners have already presented posters and given presentations about their work as part of OSSSU. It is no exaggeration to say that the advent of the Internet, with the sudden ease of communication, has made international projects such as OSSSU possible. A key component of any project in the 21st century is the design and setting up of a website, both to raise the profile of the work being undertaken and provide information to a wider

audience. The OSSSU website can be found at <http://ossu.org>.

The publication *Growing Orchids from Seed* was written with the express purpose of making basic seed sowing techniques available to a wide audience (Seaton & Ramsay, 2005). Not only can the book serve as a basic laboratory manual, the methods described require a minimum of laboratory equipment and are therefore suitable for use where resources are limited. Because a significant proportion of OSSSU partners are in the orchid-rich countries of Latin America, OSSSU has sponsored the translation of the information into Spanish, and the book is available as *Cultivo de Orquídeas por Semillas* (Seaton & Ramsay, 2009). Ideally the information should eventually become available in a wider range of languages. As a first step in this direction, *Growing Orchids from Seed* has also been translated into Chinese.

At the outset of the project two people at each institution were funded to attend the workshops in an attempt to avoid the possible loss of training/expertise if one of the individuals so trained left the institution. The original personnel have in turn cascaded their training and expertise to a large number of students and permanent staff who are presently engaged in testing the viability of the stored seed at regular intervals. We are seeking answers to a number of important questions. Seed of some orchid species are undoubtedly shorter-lived in storage than others (Pritchard *et al.*, 1999), and genera such as the South African *Disa* (Thornhill & Koopwitz, 1992) and the Central and South American *Stanhopea* and *Coryanthes* retain a reputation for being short-lived. Is this deserved? Are seeds of some genera shorter-lived than others? If this is the case, should we be considering additional storage at -196 C using liquid nitrogen?

Orchid seed-banking alone, however, cannot be the answer to all of our conservation problems. The obvious question then becomes: what is the purpose of storing orchid seed? One valuable spin-off of OSSSU is that the germination testing inevitably produces seedlings, and these can be used to enhance living collections both for research and educational purposes. In addition, seedlings can potentially be used as part of re-introduction projects.

An example of how OSSSU can contribute to orchid restoration projects is provided by a project

currently underway in Cali, Colombia (for a detailed account of the project see Seaton & Orejuela, 2009). Here groups of dedicated conservationists from different backgrounds are working together to produce an effective integrated rescue package to conserve, propagate, and re-introduce *Cattleya quadricolor* Lindl. (syn. *C. chocoensis* Linden). This involves members of the Asociación Vallecaucana de Orquideología, Jardín Botánico de Cali, Reserva Natural de Yotoco, Parque Nacional de los Farallones, and the Seed Conservation Department of the Royal Botanic Gardens, Kew.

In response to increasing concern about their status in the wild, an Action Plan has been formulated for the conservation of all *Cattleya* species in Colombia (Niessen & Calderón, 2002). Included in the Action Plan was an evaluation of the status of the remaining wild populations, the production of distribution maps, evaluation and enhancement of *ex situ* collections, and the reintroduction of endangered species. *Cattleya quadricolor*, a Colombian endemic, has been classified as Endangered (EN) (http://www.iucnredlist.org/static/categories_criteria) using the the latest IUCN Red List categories (Calderon-Saenz, 2007). Although remnant populations remain in dry and transitional humid forests from 600 to 1500 meters above sea level along a narrow strip of territory along the Rio Cauca, extensive habitat destruction and past overcollection have led to the current precarious status of the species.

In June 2005, a study group from a wide range of backgrounds was created within the Asociación Vallecaucana de Orquideología in Cali with the aim of undertaking an indepth study of the different native Colombian orchid genera. As part of the project they decided to undertake the *in vitro* production of orchids in danger of disappearing from the wild, including *C. quadricolor*, with the long-term aim of reintroducing them once more into their natural habitats.

The OSSSU project transferred seed storage tubes and some funds to the Jardín Botánico de Cali to assist with the purchase of a small chest freezer, where dry seed of Colombian orchids, including *C. quadricolor*, can be stored, both to act as an insurance policy against future losses of wild populations and to provide material for use in future conservation projects. Germination of stored seed is currently being monitored at regular intervals, thereby contributing to

an OSSSU database. It is anticipated that germination testing will continue beyond the end of the project period. The first 500 plants are currently growing with the aim of reintroducing *C. quadricolor* to sites that are protected from collectors.

However desirable this may be thought to be, from the outset it was not the stated aim of OSSSU to target seed of endangered orchids specifically nor collect orchid seed from the wild. The initial focus was to use plants under cultivation in living collections, cross-pollinating different clones where possible. With the notable exception of some countries such as Colombia, there remains a lack of information about the status of the majority of orchid species in the wild, and few are to be found on the current Global Red Lists (Pimm, 2005). It can therefore be difficult to make informed decisions about which orchid species should be priorities in terms of seed banking. Given the tiny amount of space required to store an individual orchid seed collection, it would seem sensible to store whatever we are able at present and generate as much data as practicable.

The decision to use existing collections was pragmatic. Collecting seed from the wild can be time-consuming and expensive and requires permission from the appropriate authorities before embarking on such a program. OSSSU does not, however, exclude the use of wild-collected material. In Chile, for example, few of its native species are in cultivation, and the project is indeed focusing on wild-collected seeds. In this instance it is particularly important to develop suitable symbiotic and asymbiotic germination techniques.

The overall strategy is one of capacity-building – the setting up a self-sustaining network of orchid seed banks around the globe for the future – and of gathering and collating data on a wide range of orchid species, thereby providing a sound foundation and knowledge base for future orchid seed bankers and biotechnologists. The project is already expanding to include a number of Associate Members who, at the time of writing, include additional institutions in Brazil and China (including Hong Kong), plus institutions in Europe, the first to join being Estonia, Italy, and Spain (Mallorca). Beyond OSSSU, as the network expands, an updated target has been set to have a minimum of 1000 species in storage, thereby making a significant contribution to achieving GSPC target 8.

Conclusion

Thus far, OSSSU has facilitated the establishment of a global network of orchid seed banks. In addition to storing seed according to a common protocol, the project has generated data on seed-capsule ripening times, seed numbers per capsule, media preferences for more than 250 species, the performance of more than 250 species on a single growth medium (Knudson C), and information on the relative longevity of orchid seeds representing species from a wide range of habitats. The next step is to publish these data and make them available as a resource for horticulturalists and conservationists wishing to store orchid seed and regenerate plant material from that seed. Our long-term aim is to expand the orchid seed banking network to include additional countries and institutions and establish a Global Orchid Facility that brings together the information garnered from OSSSU with the information already available but dispersed throughout the orchid literature and make this available through a dedicated website.

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FITNESS LANDSCAPES IN ORCHIDS: PARAMETRIC AND NON-PARAMETRIC APPROACHES

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ABSTRACT. Natural selection and genetic drift are the two processes that can lead to cladogenesis. Without a doubt the great diversity and floral adaptation to specific pollinators are likely consequences of natural selection. Detecting natural selection in the wild requires measuring fitness advantage for specific characters. However, few published orchid studies demonstrate that floral characters are influenced by natural selection. If selection is temporal or weak, then this may explain why we rarely find selection on floral characters. Alternatively, selection on a character may not follow commonly used mathematical models that are based on linear, disruptive, and stabilizing selection and serve as null models. Moreover, fitness advantages are usually tested on general models, which assume that the parameters are normally distributed. If we forego the idea that selection follows specific mathematical models and Gaussian distribution and that all types of selection landscapes and other types of distributions (binomial, Poisson) are possible, we may discover evidence that the process of selection does play a role in explaining the great diversity of orchids. Here I show and compare the use of traditional and non-parametric approaches for measuring selection of floral characters. I hypothesize that many characters are likely to be influenced by selection but, using traditional approaches, will fail to observe selection on the measured characters, whereas non-parametric approaches may be more useful as a tool to detect selection differences among characters.

RESUMEN. La selección natural y la deriva genética son dos procesos que pueden conducir a la cladogénesis. Sin duda, la gran diversidad y adaptación floral a polinizadores específicos es sorprendente y es una consecuencia de la selección natural. La observación de la selección natural en el medio ambiente silvestre requiere el medir la ventaja de su idoneidad para ciertos caracteres específicos; sin embargo, hay pocos trabajos científicos publicados que apoyan la idea de que los caracteres están bajo la influencia de la selección de caracteres florales en las orquídeas. Una de las razones que podría explicar porqué raramente identificamos la selección en caracteres florales es que la selección puede ser temporal. Una hipótesis alternativa es que la selección de caracteres podría no seguir los modelos básicos que se basan en selección lineal, disruptiva, y estabilizadora. Estas ventajas de idoneidad son usualmente puestas a prueba con modelos generales que asumen que los parámetros están distribuidos en forma normal. Si nos olvidamos de la idea de que la selección sigue tal tipo de distribución Gaussiana y que todos los tipos de panoramas de selección son posibles, podríamos descubrir evidencia de que el proceso de selección sí tiene un rol en la explicación de la gran variedad de orquídeas. Aquí demuestro y comparo el uso del enfoque tradicional y no-paramétrico para medir la selección de caracteres florales con ejemplos de *Tolumnia variegata*, *Lepanthes rupestris*, y *Caladenia valida*.

KEY WORDS: orchid flowers, natural selection, fitness advantage, mathematical models

Evolution is a consequence of random (genetic drift) or non-random processes (natural selection). Natural selection requires the presence of variation, heritability of the variation, and fitness differences among individuals in a panmictic population (Endler,

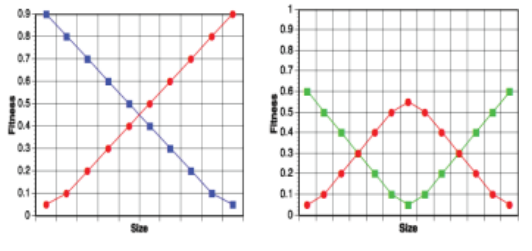
1986). When we discuss fitness in an evolutionary context we are referring to the ability of an individual to leave viable offspring. However, in most cases the ability to measure the number of such offspring in the next generation is often limited because of the difficulty

in monitoring individuals throughout their lifespan. For example, to evaluate the true lifetime fitness of an orchid would require monitoring orchid seeds, which cannot be seen or followed in the wild (in most cases) or assigned to specific parents in a population unless genetic markers are used.

Consequently, fitness is measured through surrogate indices, such as number of flowers, number of pollen (pollinaria) removed and deposited on the stigma, number of fruits, number of seeds, length of the life span, etc. It is assumed that the number of flowers and pollinaria removed or deposited will be positively correlated with number of fruits. The number of these fruits, in turn, is likely positively correlated with the number of seeds produced and ultimately the number of viable offspring sired. In the same way, it is assumed that life span is positively correlated with lifetime reproductive success and that longer-lived individuals will produce more offspring. These surrogate variables of fitness have been shown to be correlated with evolutionary fitness.

Traditionally the models of natural selection that have been described follow a linear (positive or negative) or quadratic relationship (stabilizing and disruptive selection) between the character of interest and the fitness index (Box 1: Kingsolver *et al.*, 2001). These relationships are used as null models for testing if a fitness advantage among morphologically different individuals is present. Such models are based on the idea that natural selection follows mathematical equations. Consequently, they require assumptions on how natural selection functions, the most serious of which is that phenotypic and natural selection follow pre-established mathematical equations. In a simple example, let us assume that plants with larger inflorescences have higher fitness (fruit set). A model of selection built from a linear equation would predict that selection should result in larger and larger inflorescences. However, biological limitations are likely to be present; perhaps large inflorescences do not attract more pollinators than intermediate inflorescences so that an asymptote should be reached. The advantage for large inflorescences may be tempered if high production of flowers and fruits results in high energetic costs that negatively affect the likelihood of future mating events.

Box 1. Traditional models of selection for linear (POSITIVE and negative) selection, stabilizing, and disruptive selection. Linear models test the possibility of either the small or large form of a character having a selection advantage following a linear equation. The quadratic equation for stabilizing selection tests if the medium-size character has an advantage over the small and large sizes. The quadratic equation for disruptive selection tests if small and large sizes have advantages over the intermediate-size character.



Phenotypic selection

In most cases, phenotypic selection is the process being studied in the field, not natural selection, because the underlying architecture of genetic inheritance of the character in question is unknown. The assumption is that the morphological differences among individual are genetically based, which is the case for all orchid studies to date. The complexity of the genetic architecture behind morphological characters will influence how quickly the character can be inherited among generations for specific fitness differences.

Here I review approaches for evaluating phenotypic selection in the wild with examples from the orchid literature and unpublished data. I have divided the following discussion into two parts. First I show how to measure fitness differences among characters using the traditional approach (Lande & Arnold, 1983; Endler, 1986), and then I discuss a non-traditional approach to the problem of detecting phenotypic selection in the wild (Schluter, 1988).

Common regression approach

The traditional approach is described in detail by Lande and Arnold (1983; see also Endler, 1986 for a simpler description of the mathematics). They showed how to use multiple regression analyses of untransformed character traits that are regressed on the relative measure (standardized by the mean) of fitness

estimates to quantify the strength of selection. The analysis is applied in two (or three) steps. The first step is estimating the slope of the linear regression line (defined as the selection gradient), which is an expression of directional selection (positive or negative). The second step is investigating the quadratic regression (multiplying the trait character as follows: $[(T_{i1} - \text{mean } T_1)^2]$, where T_1 is the mean of the trait being investigated and T_{i1} is the size of the trait for each individual) and regressing this on the standardized fitness. The non-linear selection coefficients estimate the presence of stabilizing (selection coefficient is negative) and disruptive selection (selection coefficient is positive). In addition, the effect of interaction among traits ($T_{12} = T_1 \times T_2$; where T_2 is the size of another character of interest for the same individual) can be evaluated. However, I will not discuss the effect of interactions among traits in this paper. See Boxes 2 and 3 for examples of how to calculate the above.

Selection differentials and selection gradients — The concept of differentials is to define the difference between the population mean before and after selection. This should not be confused with a selection gradient, which is the expected relative fitness of a specific trait compared to another trait at a specific time and space.

Indices of fitness — There are three types of indices of fitness commonly used. The simplest is the categorical index of presence or absence (such as alive or dead) or individuals that produced fruit or not. In this approach, individuals have an all-or-none response. The analysis used in this case is a logistic regression, where the proportional response is plotted as a consequence of the continuous variable.

The fitness variable can also be the number of units, such as the number of flowers, fruits, seeds or the length of the life span. However, a relative measure of fitness is the most common index used and is defined as the fitness of an individual over the mean fitness of the population. Using this definition, an individual with a relative fitness of 1.00 would be equal to the mean fitness of the population, whereas a fitness of 0.5 represents an individual with a fitness just 50% of the mean, and an individual with a relative fitness of 2.00 produced twice as many offspring as the mean. These data are most often plotted as a response to a continuous variable using regression analyses.

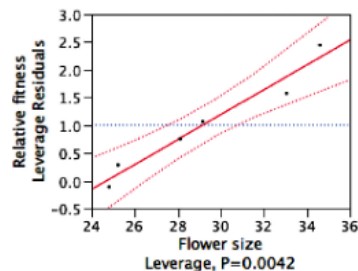
Box 2. Example of calculations of basic parameters for a hypothetical orchid in a population of 6 individuals, assuming T_1 = flower size, fitness index = number of fruits. Note that the mean of the relative fitness index will always sum to 1.00.

Sample	T1= Flower size	(T1-mean T1)2	Fitness index = number of fruits	Relative fitness index = number of fruits/mean number of fruits
Ind. 1	24	26.294	0	0
Ind. 2	25	17.361	1	0.3158
Ind. 3	29	0.028	2	0.6316
Ind. 4	30	0.694	3	0.9474
Ind. 5	33	14.694	5	1.5789
Ind. 6	34	23.361	8	2.5263
Mean	29.16		3.167	1.0000

Box 3. The regression analysis. In this case I use common statistical software to test for linear, disruptive, and stabilizing selection. The data are from Box 1. Analysis performed with the statistical program JMP. A selection of the test results shows a partial table with statistical values; a $p < 0.05$ is considered significant in ecology. We observe that $p = 0.0042$ for flower size, so we reject the null hypothesis and accept that there is a relationship between flower size and fruit set. The quadratic function is not significant here.

	t-ratio	Prob > t
Intercept	-6.77	0.0066
Flower size	7.94	0.0042
(X2 - mean X) of flower size	2.26	0.1087

The figure shows the relationship between flower size and relative fitness. The solid red line shows the mean and stippled lines the 95% confidence interval of the line. The line is calculated (estimated) as $-5.208 + 0.213 \times \text{flower size}$. Note that the line is the best estimate of the relationship between the two continuous variables.



Assumptions of the regression analysis — Regression analysis has a number of assumptions. For example, the traditional linear model assumes five conditions: 1) for every size of some character (x-axis) there is a population of response that follows a normal distribution; 2) across the size variable (x-axis) the variance is equal, so that there is homogeneity of variance for each x; 3) the mean of y values falls in a straight line with all the other means of y values; 4) when the data are collected, individuals are selected at random; and 5) there is no error in the measurement of x. These are the conditions for testing if there is a linear relationship among two variables. If the quadratic function is the null hypothesis, then the relationship must fit that equation (Zar, 1999).

Non-parametric approach

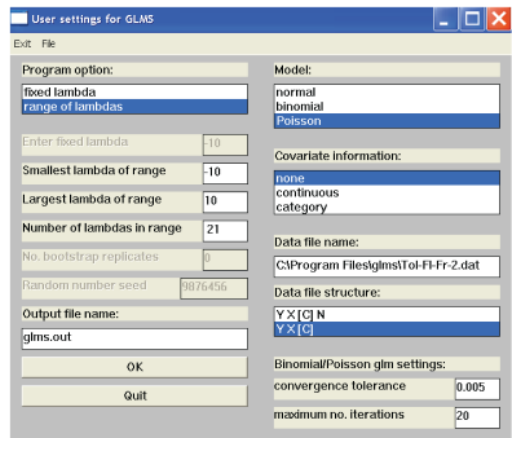
The limitation to the parametric approach is that fitness advantage may not fit the null mathematical equations. In other words, the fitness landscape may be some other function that does not follow a linear or quadratic equation. An alternative approach is to evaluate the best possible fit of the data to equations using a cubic spline approach and allow the data to inform us of the best-fit line. The objective is to construct models of relationship of the explanatory variable that best describes the response variable. Using this approach we do not assume that relationship between x and y follows a specific mathematical equation (linear, quadratic, etc). This method, which has been applied to evolutionary models by Schluter (1988), Schluter and Nychka (1994), and Tremblay *et al.* (2010), is a two-step process and can be applied using Windows software GLMS, developed by Schluter and Nychka (1994) and found on Schluter’s website (<http://www.zoology.ubc.ca/~schluter/software.html>).

The first steps are to determine the best level of complexity of the equation. A large range of possible lambda and two indices of the fit of the complexity of equation are used, OCR and GVC scores (Schluter, 1988). One chooses the lowest value and applies this value for determining the relationship between the explanatory variable (phenotype) and fitness response (e.g. fruit set). The mathematics behind the application of the cubic spline is complex and not the goal of this paper. Those interested should search the references cited above as well as cubic spline on the Internet for an

Box 4. Application of the program GLMS from Dolph Schluter (University of British Columbia). Step 1: The data are entered in a text file and saved as a document with the .dat extension. Fitness data (number of fruits) are entered in the first column, and the morphological character is entered in the second column.

Example of data entry

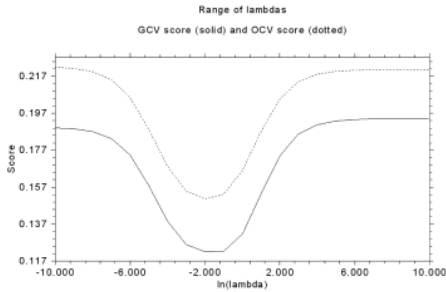
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0 24
1 25
2 29
3 30
5 33
8 34
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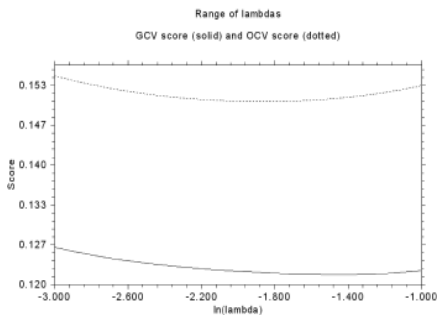
introduction to the concept. The process of performing the analysis is presented in Boxes 4-7.

Caveats of the cubic spline approach — The down side of this non-parametric approach using cubic spline for determining the best line is that we do not have a null model for how to test the observed data. An additional limitation is that environmental effects can result in biases due to environmental covariances between traits and fitness (Rauscher, 1992), although this is applicable to both methods. The challenge is detecting when a specific factor of the environment influences not only the phenotype but also the fitness of that phenotype. For example, let us consider a hypothetical epiphytic orchid. When the orchid is growing in a section of a tree where the substrate is decomposing, nutrient availability is likely to be greater. So plants may produce more flowers or larger flowers, which increases fruit or seed production if fitness is influenced by number of flowers or size. In this scenario there

Box 5. Determining the best lambda will determine how complex the line must be to explain the data. Step 2: Starting with a range of lambda from -10 to 10, run the program. Notice that both the GVC and OCV score dip in the range of -2.00, indicating that best lambda to explain the data is in this area. Re-run the program choosing a range of lambda from -3.00 to -1.00.



Notice that minima are now in the range of -2.2 to -1.4, depending on which index is evaluated. To evaluate the difference between the two indices, it is recommended that you evaluate both minima. Let us choose -1.6 for the following steps. In general, the differences between the two scores (thus the ranges) have minimal effect on the ultimate outcome.

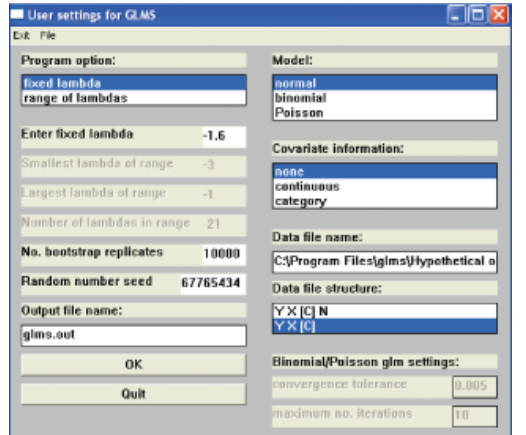


is an environmentally induced covariance between substrate quality and fitness. The size of the plant is an interaction between genetics and the environment. The plasticity of expression in orchids can be dramatic as a response to resources, to the point that sex expression can vary (Zimmerman, 1991).

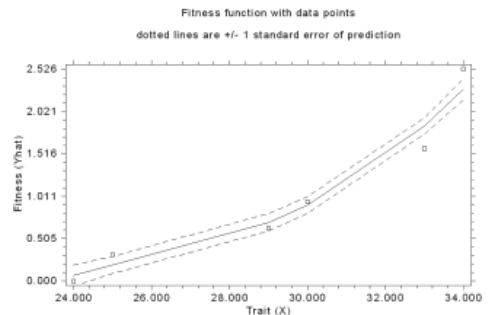
Examples of phenotypic selection in orchids

Cintrón-Berdecia and Tremblay (2006) showed that selection on column length of *Lepanthes rupestris* Stimson was likely present in some populations at certain time periods and that there was significant effect when all data were pooled across

Box 6. Constructing the relationship between fitness and character variation. Step 3: We apply a fixed lambda of -1.6. Then we choose the number of bootstrap replicates (to calculate the error around the best line). Choose a bootstrap of 1000 or more.

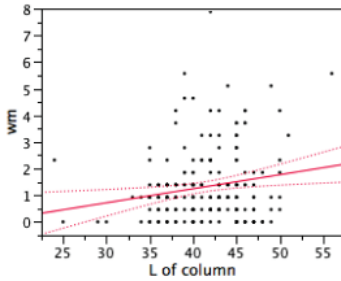


Box 7. Step 4: Observation of the fitness landscape for the trait under study. Note that the line is not straight as predicted by the linear model. The rate of change as a consequence of size varies across the morphological landscape. Our hypothetical orchid with a character trait size of 30 has a fitness advantage of 1 (the mean of the population), whereas a plant of size 32 has 1.5 as its fitness advantage. In other words, it is expected to have 50% more progeny than a plant of size 30, and an individual with a size of 29 would be expected to have half the fitness of an individual of size 30.

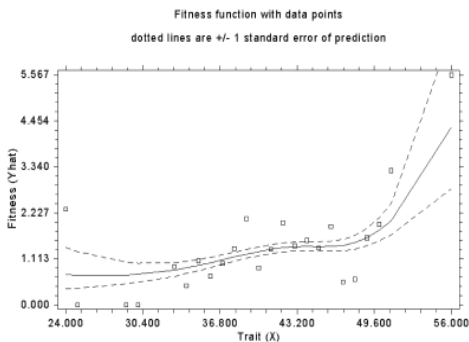


all populations. They showed that larger columns had selective advantage over shorter columns (selection coefficient, $s' = 0.053$, $p < 0.05$), where the probability of having the pollinaria removed is higher. If we re-evaluate this set of data using the non-parametric approach, we do show a disadvantage

Box 8. Comparison of traditional and non-traditional approaches to detecting fitness advantage in *Lepanthes rupestris*. Data from Cintrón-Berdecia and Tremblay (2006). The linear relationship between column length and pollinaria removal, w_m (relative fitness). $n = 197$, $w_m = -.086 + 0.053 * \text{Length of the column}$, $r^2 = 0.023$, $p < 0.01$. Each point represents an individual, and many individuals overlap.



The non-parametric evaluation of the relationship. Short column length has a selective disadvantage, and longer column length is associated with a large increase in the probability of pollinaria removal. The relationship does not follow a simple linear function. Each point represents the mean fitness for the trait of a specific size. Note also that the 95% confidence interval varies as a consequence of sample size and consistency in the response variable. Squares represent the mean of the response for the trait of a specific size.



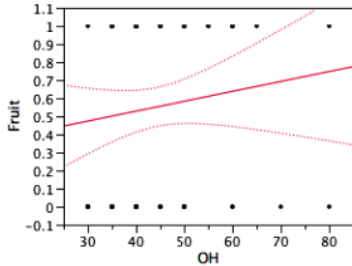
of small columns over larger columns; however, the fitness landscape is not linear, and larger plants have significantly larger probabilities of having their pollinaria removed (Box 8). In another study (Tremblay, 2006), pollinaria removal was shown to be influenced by flower size, but flower size is in turn dependent on the position of the flower on the inflorescence as is column length. Flowers at the

base of inflorescences were larger and may represent a size more adapted to the pollinator (presumably a black-wing fungus gnat). Nevertheless, many plants had much smaller flowers, which appear to be poorly adapted for pollinaria removal.

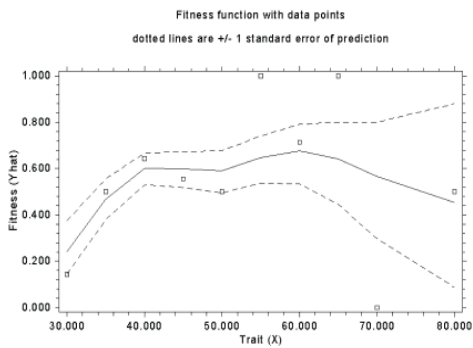
Caladenia valida (Nicholls) M.A.Clem. & D.L.Jones, a widely distributed species in the Australian states of Victoria and South Australia, usually bears a single flower. Consequently, the response variable for evaluating fitness is a categorical variable of fruit or no fruit. Here I evaluate the effect of flower size on fruit set. The character measured is the size of the flower from the tip of the lateral sepal to tip of the petal, a possible measure of what the pollinator may see from far away. It is also assumed that the tips of the sepals have osmophores, and thus having these extended may result in a more extensive odor plume (Salzmann *et al.*, 2006; Faast *et al.*, 2009; Phillips *et al.*, 2009). The linear model would indicate that no effect of flower size is present ($p > 0.05$), even though we observed a slight slope showing that fruit set increases with flower size, but the 95% confidence intervals are large, and no pattern could be confidently detected. Alternatively, the cubic spline approach shows that small flowers (< 40) have much lower probability of setting fruit, whereas larger flowers (> 40) have more or less the same probability of setting fruit, indicating a plateau has been reached with no selection detected among in size classes 40+.

Tolumnia variegata (Sw.) Braem, a twig epiphyte, is the most widespread species of the genus with a distribution from the Virgin Islands in the eastern Caribbean westward to Puerto Rico, Hispaniola, Cuba, and the Cayman Islands. Plants commonly occur on shrubs and small trees mostly in secondary habitats and in dry to wet regions from near sea level to 800 m (Ackerman, 1995). Plants at Tortuguero, Puerto Rico, flower mainly from August to December, but it is not uncommon to see a few plants with flowers throughout the year (Ackerman, 1995). The orchid is self-incompatible, offers no pollinator rewards, and is severely pollination-limited (Ackerman & Montero Oliver, 1985; Calvo, 1993; Sabat & Ackerman, 1996). Pollinators are female *Centris decolorata* Lepeletier (Apidae; Sabat & Ackerman, 1996). I evaluated the relationship between flower display (total number of flowers produced) and reproductive success. Here the

Box 9. The first graph shows the relationship using a logistic regression of overall flower size with the probability of fruit set. No significant effect of size on fruit set is detected, as $p > 0.05$.



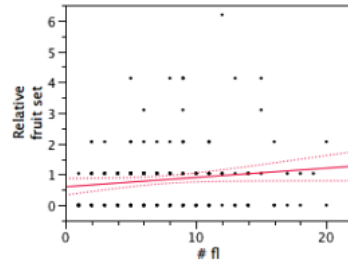
The non-parametric evaluation of the relationship. Short column length has a selective disadvantage, and longer column length is associated with a large increase in the probability of pollinarium removal. The relationship The non-parametric analysis shows that small flower size (<40) is a disadvantage in setting fruit, whereas a flower size of 40-80 has the same expected fruit set. Note the 95% confidence interval is large for flower size of 50+, and the true trend cannot be predicted with confidence. This pattern would be impossible to detect using the traditional approach.



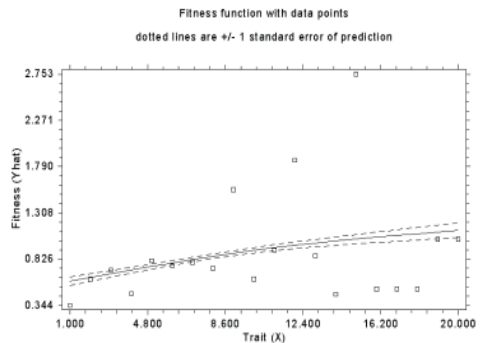
fitness variable is a Poisson distribution, but because not all individuals had the same number of flowers, I used the relative fitness index (as explained above). It has been shown previously that larger display size can result in higher fruit set in this orchid (Sabat & Ackerman, 1996) and other orchids (Huda & Wilcock, 2008).

The parametric and non-parametric analysis had similar results (Box 10). The error around the line is smaller for the cubic spline analysis, and the relationship is not linear (although not far from it). In general, the results from these two analyses are similar

Box 10. The linear relationship in *Tolumnia variegata* between number of flowers and relative fruit set. Relative fruit set = $0.599 + 0.0302 * \# \text{ fl}$, $F = 3.557$, $r^2 = 0.01$, $p = 0.061$. Note that the equation explains only 1% of the variation.



The cubic spline analysis of number of flowers and fruit set. Squares represent the mean fitness for the trait. The relationship is similar to the linear regression but shows a tapering off when plants have many flowers.



enough to support either method. The cubic spline analysis has the drawback that the estimated means for large traits do not fall within the 95% confidence interval and are highly scattered. I would recommend increasing sample size for these traits.

Discussion of methods

The cubic spline approach of evaluating phenotypic selection is another tool available to evolutionists for exploring possible fitness landscapes and determining if some phenotypes have an advantage over others. If patterns are observed, then two of the conditions for natural selection must be present (variation, fitness difference). The next steps would be to determine whether variation among individuals is heritable and, if so, how variation is inherited. When patterns of phenotypic selection are observed using the traditional

approach, the cubic spline results will likely be similar. The limitation of using cubic spline is that no null hypothesis is present, and so a strong *a priori* view could bias interpretation of the results. In both approaches, small sample size will be an impediment, and results should be evaluated with caution. Fitness variation among phenotypes is not likely to be limited to linear and quadratic equations. If we restrict our analyses to only these equations, then we undermine the whole diversity of possible natural selection scenarios.

Evaluating a selection coefficient for an individual is likely to be more complex than the methods used here. Phenotypic plasticity, for example, can have a strong influence. Flower size of *Caladenia valida* can change from one year to the next as a consequence of environmental variation and genetic architecture, so the likelihood of setting fruit is not fixed for individuals and possibly changes as a result of size differences (Tremblay *et al.*, 2010).

Why are we not finding much evidence of phenotypic selection in orchids, even though we observe so much apparent adaptation to different pollination syndromes? One of possibilities is that our tools have been inappropriate. Naturally, it is also likely that selection is periodic (Schemske & Horvitz, 1989) and absent for many of the variables we are measuring. Moreover, some changes may be the consequence of small effective-population sizes, resulting in periodic evolution by genetic drift (Kingsolver *et al.*, 2001; Gravilets, 2003; Tremblay *et al.*, 2005).

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**PROCEEDINGS OF
THE THIRD SCIENTIFIC CONFERENCE
ON ANDEAN ORCHIDS**

POSTERS

*CONSERVATION SCIENCE
ECOLOGY
SYSTEMATICS*

**APLICACIÓN DE LA TÉCNICA DE ENCAPSULACIÓN – DESHIDRATACIÓN
PARA LA CRIOCONSERVACIÓN DE SEMILLAS Y PROTOCORMOS DE
ONCIDIUM STENOTIS (ORCHIDACEAE)**

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El presente trabajo pretende desarrollar un sistema que posibilite la crioconservación de semillas y protocormos de *Oncidium stenotis* en nitrógeno líquido (-196 °C), con el uso de la técnica de Encapsulación-Deshidratación. La primera fase del proyecto consistió en tomar dos fracciones de las semillas, una de ellas fue puesta a germinar en medio Knudson líquido adicionado: ácido α -naftalén acético y ácido giberélico y la segunda fracción en medio Knudson sólido (7 gL⁻¹ de agar) enriquecidos con: benziladenina, ácido indolacético, ácido α -naftalén acético y ácido giberélico. Ambas fracciones se incubaron a 23 \pm 2 °C y con un fotoperíodo de 12 horas luz. La adición de diferentes concentraciones de reguladores de crecimiento en ambas fracciones se la realizó con el fin de acelerar el proceso de

germinación. La fase que se realizará posteriormente es encapsular las semillas y protocormos en alginato de sodio al 3%, las cápsulas posteriormente serán deshidratadas en concentraciones crecientes de sacarosa 0.15 M (24 h), 0.25 M (24 h) y 0.5 M (24 h) para luego ser colocadas en sílica gel por 5 horas. La crioconservación se efectuará por inmersión directa en nitrógeno líquido durante una hora. El descongelamiento se realizará a baño maría (37 °C) por un minuto, luego las cápsulas que contengan semillas se sembrarán directamente y los protocormos se recultivarán sin las cápsulas en medio Knudson sólido, con la adición de diferentes concentraciones de Benziladenina e Ácido Indolbutírico, para buscar el medio adecuado de resuperación post-congelamiento.

ECOLOGY

**MATING SYSTEM AND FEMALE REPRODUCTIVE SUCCESS OF
THE ENDEMIC, EPIPHYTIC *PROSTHECHEA* AFF. *KARWINSKII*
(ORCHIDACEAE)**

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Prosthechea aff. *karwinskii* (Mart.) Soto Arenas & Salazar (unpublished name) is an endemic Mexican epiphytic orchid with a restricted distribution. It occurs in deciduous oak forests, particularly on *Quercus deserticola* in the northern portion of Michoacán state. Due to their ornamental uses, this and other orchid species have been heavily collected from their natural populations. Learning about the reproductive biology of this species is important, as we may gain knowledge about the probable repercussions of overcollecting on their genetics and ecology. This will be a useful tool for the management and conservation of the species. The objectives were to 1) study the mating system of *P. aff. karwinskii*, 2) determine if the species requires an external pollinating agent for sexual reproduction, and 3) evaluate female reproductive success of this species. Exclusions (150) were

made before anthesis. Once the flowers opened, we applied four pollination treatments: manual selfing, outcrossing, spontaneous autogamy, and open pollination. We subsequently quantified the production of fruits and viability of the seeds. Preliminary results showed that outcrossing and selfing treatments exhibited statistically higher fruit set than spontaneous autogamy and open pollination treatments, meaning that *P. aff. karwinskii* has the capability to reproduce via self-pollination. *Prosthechea* aff. *karwinskii* is pollinator-dependent for sexual reproduction, taking into account that the spontaneous autogamy treatment showed nil fruit production. Female reproductive success in open pollinated flowers was lower compared to fruit set of hand pollination treatments. This indicates that this species, as other species of tropical orchids, is pollination-limited.

HONGOS ENDÓFITOS DE LA ORQUÍDEA EPÍFITA *LAELIA SPECIOSA*

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Las orquídeas mantienen en la naturaleza estrechas relaciones con hongos. Durante el trabajo con la reproducción *in vitro* de *Laelia speciosa* observamos al microscopio que semillas y plántulas presentan infestación de hongos en diversos tejidos. También se analizaron plantas adultas colectadas en el campo. Para identificar a los hongos e inferir el papel de su presencia en la planta secuenciamos su ADN. Se extrajo ADN de tejidos esterilizados superficialmente como raíz, hoja, cápsula y semillas; luego se amplificó la región del ITS ribosomal con los *primers* ITS1F e ITS4. Posteriormente se clonaron los fragmentos con TOPO TA4 y se secuenciaron usando la química de Big Dye terminator en un ABI 3100. En total se secuenciaron más de 150 clones positivos, de éstos el 50% correspondieron a secuencias de *L. speciosa*, aunque

los *primers* empleados en teoría son específicos para hongos. El resto de las secuencias agrupadas en 97% de similitud correspondieron a 19 especies de hongos de los géneros *Alternaria*, *Cylindrocarpon*, *Curvularia*, *Fusarium*, *Myrmecridium*, *Neonectria*, *Penicillium*, y *Tetracladium*, así como a especies desconocidas de los Helotiales, Lasiosphaeriaceae, Nectriaceae, Pucciniomycotina, Sordariomycetes, y Tricholomataceae. Muchos de estos taxa están presentes en las semillas, lo que sugiere que estos hongos llegan a éstas vía placenta. En algunos casos, hongos parásitos, causan la muerte de las semillas y en otros casos, hongos micorrízicos, facilitan su germinación. La diversidad de hongos endófitos en *L. speciosa* es considerable y la biología de los organismos involucrados es diversa y va desde los parásitos y saprobios hasta los micorrízicos.

EVIDENCE OF PROTANDRY IN *Aa* RCHB.F. (ORCHIDACEAE, CRANICHIDEAE)

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Protandry has been described in orchids since Darwin (1862) as a strategy to promote cross-pollination. Three different forms of protandry have been reported in tribe Cranichideae: downward movement of the labellum in *Spiranthes* species, upward movement of the column in *Sauroglossum elatum* Lindl. and *Manniella* spp., and downward movement of the column in *Prescottia stachyodes* (Sw.) Lindl. In the present work, through longitudinal and transverse sections of flowers of different developmental stages, we reported column movement of *Aa erosa* (Rchb.f.) Schltr. and the mechanism responsible for

the movement. Our result shows that in an early flower stage (male phase), the column of *Aa erosa* is straight. The gradual cell death of the dorsal side of the column and size increase of the cells of the ventral side cause the column to bend downward to almost 90° representing the female stage. Some authors have suggested self-pollination in *Aa*. But flies exploring inflorescences of *Aa* species have been observed in the field by the author. This observation plus the evidence of protandry in *Aa erosa* could discard self-pollination as the only strategy of pollination in this genus.

SYSTEMATICS

**A PHYLOGENETIC ANALYSIS OF THE GENUS *PLEUROTHALLIS*,
WITH EMPHASIS ON *PLEUROTHALLIS* SUBSECTION *MACROPHYLLAE-
FASCICULATAE*, USING NUCLEAR ITS AND CHLOROPLAST DNA
SEQUENCING**

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Several revisions of the genus *Pleurothallis* have been proposed. Luer has proposed that *Pleurothallis* species in subgenus *Scopula* be segregated into the genera *Colombiana* and *Ancipitia*. Szlachetko and Margonska (2001) proposed the genus *Zosterophyllanthos* for *Pleurothallis* subsection *Macrophyllae-Fasciculatae*. As an alternative, Luer (2005) proposed the genus *Acronia* by uniting *Pleurothallis* subsection *Macrophyllae-Fasciculatae* with subsections *Acroniae* and *Amphygiae*. The molecular phylogenetic studies by Pridgeon and Chase (2001), however, suggested that these taxonomic revisions might not be justified. We report here a more detailed phylogenetic analysis of the

genus *Pleurothallis*, with emphasis on subsection *Macrophyllae-Fasciculatae*, with data primarily from nuclear ITS sequencing, supplemented with preliminary data from plastid DNA (*rpoB2*, *rpoC1*, and *ycf1*) sequencing. Some initial, tentative conclusions can be drawn. In the strict consensus maximum-parsimony tree of ITS data, many of the clades collapse, leaving a polytomy with a single, highly supported node that tentatively could be used to delimit the genus *Pleurothallis*. Such a tree would argue for an expanded concept of the genus *Pleurothallis*, in which the groups *Ancipitia*, *Colombiana*, and *Acronia/Zosterophyllanthos*, if shown to be monophyletic, are relegated to subgenera.

PHYLOGENETIC ANALYSIS OF THE ANDEAN GENUS *BRACHYCLADIUM* LUER (SYN. *OREOPHILUS* HIGGINS & ARCHILA) AND CLOSELY RELATED GENERA BASED ON NUCLEAR ITS SEQUENCING

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We previously reported that nrITS sequencing and phylogenetic analysis revealed at least four cryptic species within *Brachycladium nummularium* (syn. *Oreophilus nummularius*). Sequencing of additional species in the genus revealed two major clades within *Brachycladium*, the “nummularium”-complex plus *B. stalactites* and Luer’s section *Amplectentes*. Since the relationship of *Brachycladium* to *Andinia* had been noted earlier, a number of *Andinia* species were also included. Two clades were observed, a clade containing *A. lappacea*, *A. pensilis*, and *A. vestigipetalata* that was closely related to *Brachycladium* and a second, more distantly related clade containing *A. dalstroemii*, *A. pogonion*, and *A. schizopogon*.

Samples of two additional genera, *Masdevalliantha* Szlachetko & Margonska and *Xenosia* Luer, were included in the analysis based upon morphological similarity to *Andinia*. These two genera formed a clade that was sister to both *Brachycladium* and *Andinia*. Only the node subtending clades of all four genera had a high level of bootstrap support, whereas nodes subtending clades of *Brachycladium* plus *Andinia*, or *Brachycladium* alone, had much lower support. If future chloroplast sequencing produces phylogenies congruent with nrITS, this would indicate that *Andinia*, *Brachycladium* (syn. *Oreophilus*), *Masdevalliantha*, and *Xenosia* should all be placed in the same genus.

BARCODING THE SPECIES OF *PLEUROTHALLIS* SUBSECTION *MACROPHYLLAE-FASCICULATAE*

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The long-term goal of this project is to have a genetic “barcode” for the described species in *Pleurothallis* subsection *Macrophyllae-Fasciculatae* (syn. *Acronia* Luer) to facilitate identification, recognition of new species, biodiversity assessment, and conservation of this genus in Mesoamerica and the Andes. To this end, a living collection is being assembled at Colorado College with plants from commercial operations in South America (Ecuagenera; Colomborquídeas; Orquídeas del Valle) and the U.S. (Andy’s Orchids; Hanging Gardens; J & L Orchids); and private collections in the U.S. (O’Shaughnessy). As these plants flower and identities are confirmed, photos are taken; in the future, herbarium sheets will be prepared

and flowers preserved in spirits. For some species, or from some locations such as Central America (private collection of Archila), only leaf samples have been obtained. Between living plants and leaf samples ~100 different species have been assembled, which is approximately 46% of the described species. A genetic barcode for this subsection of *Pleurothallis* will likely consist of three sequences. While only nrITS has been sequenced for these plants so far, the chloroplast sequences *rpoB2*, *rpoC1*, and the 3’ and 5’ ends of *ycf1* are currently being investigated to determine which provide greatest variability, perhaps to combine with *matK* or *trnH-psbA* as per the CBOL Plant Working Group.

