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# LANKESTERIANA

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**VOL. 13, No. 1–2**

**AUGUST 2013**

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## **PROCEEDINGS**

of the

## **FOURTH SCIENTIFIC CONFERENCE ON ANDEAN ORCHIDS**

Guayaquil, Ecuador, October 31–November 04, 2012

Edited by

**ALEC M. PRIDGEON**

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# LANKESTERIANA

INTERNATIONAL JOURNAL ON ORCHIDOLOGY

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Effective publication date: July 30, 2013

Layout: Jardín Botánico Lankester.

Cover: Inflorescence of *Catasetum tenebrosum* from Peru, with female (bottom) and male flowers (top).

Photograph by G. Gerlach.

Printer: MasterLitho

Printed copies: 500

Printed in Costa Rica / Impreso en Costa Rica

R Lankesteriana / International Journal on Orchidology  
No. 1 (2001)-- . -- San José, Costa Rica: Editorial  
Universidad de Costa Rica, 2001--

v.

ISSN-1409-3871

1. Botánica - Publicaciones periódicas, 2. Publicaciones  
periódicas costarricenses



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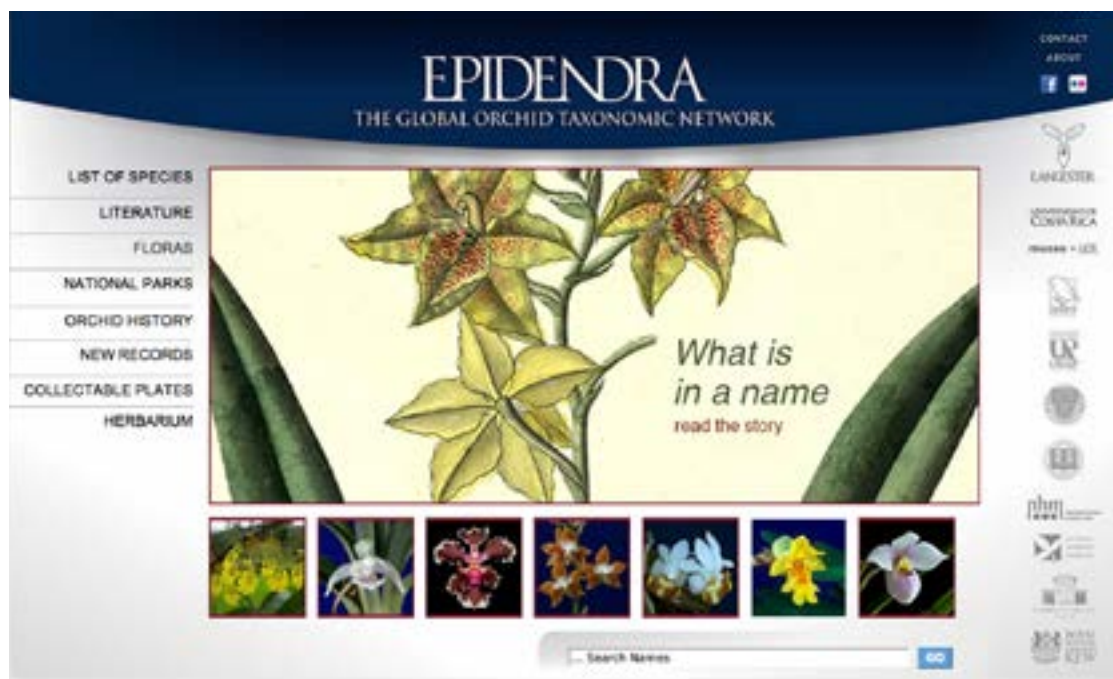
*The editors*

The Global Orchid Taxonomic Network at a click

[www.epidendra.org](http://www.epidendra.org)

Now with a new user interface, the online database on taxonomic information by Lankester Botanical Garden includes more than 7,000 orchid names, completely cross-referenced and with evaluated synonymies.

The electronic file on each one of the names accepted by the taxonomists at the research center includes *free, immediately downloadable* protologues, type images, illustrations of the original materials, historical and modern illustrations, photographs, pertinent literature and, when available, digital images of species pollinaria.



An index (under the button “List of species”) allows the users to search for any published name, independently if it is accepted or not by the taxonomic compilers. Synonyms are linked to their accepted name, where additional materials (including images) are available for download.

*Hundreds* of new species names and documents (mostly protologues), images (including high-res files), publications and other materials relative to orchid systematics, distribution and history are added to the database on a monthly basis (new entries can be searched by clicking on the “New records” button).

Since March, 2012, *new pages* are devoted to the orchid species recorded in the rich system of national parks and other protected areas in Costa Rica (“National Parks” button), updated checklists of the orchid floras of Central American countries (“Floras” button) and to interesting aspects of orchid history.

Under the button “Collectable plates”, the research staff at Lankester Botanical Garden makes available to the public the most detailed images of orchids from the collections at the Center, organized in a series of collectable plates that can be *downloaded for free*. New ones are added each week.

Supported by the University of Costa Rica and the Darwin Initiative, *EPIDENDRA, The Global Orchid Taxonomic Network* counts with the collaboration of respected taxonomists and leading botanical institutions worldwide.

## PREFACE

In November 2012, the Fourth Scientific Conference on Andean Orchids was held, seven years after the First Conference in Gualaceo, Ecuador. What began as an idea conceived at a dining room table with Pepe Portilla of Ecuagenera in 2004 is now an internationally renowned series with two goals: 1) convene experts from the Andes and elsewhere to share their knowledge and promote the protection and preservation of the unparalleled species richness of the Andes, and 2) help train students from all the Americas in techniques related to systematics, ecology, and conservation science that can be applied and shared with others in their own countries. Since the First Conference, there have been more than 100 speakers in the series from around the world with steadily increasing numbers of registrants and poster abstracts. Over 40 scientific posters were on display at the Fourth Conference, most of them from Andean nations, Mexico, and Brazil.

ALEC M. PRIDGEON

## ACKNOWLEDGMENTS

We thank the organizers of this Conference – Pepe Portilla, Marcelo Bejarano, Arcadio Arosemena, and Nicolás Romero – and the many sponsors: Ecuadorian Orchid Association, Guayaquil Botanical Gardens, Expoguayaquil, and Ecuagenera Cia. Ltda. We also thank Ecuagenera Cia. Ltda. for organizing tours and the orchid show associated with the Conference. Finally, we are grateful to Franco Pupulin and the Universidad de Costa Rica for agreeing to publish the *Proceedings* of the Conference here.

ALEC M. PRIDGEON

# MOLECULAR PHYLOGENETICS OF *MORMOLYCA* (ORCHIDACEAE: MAXILLARIINAE) BASED ON COMBINED MOLECULAR DATA SETS

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**ABSTRACT.** The Neotropical orchid genus *Mormolyca* Fenzl, as currently circumscribed, encompasses a diverse group of ca. 27 species. Many of these were included traditionally in *Maxillaria* sect. *Rufescens*, when similarity of floral morphology was considered foremost in their classification rather than the evolutionary history of the taxa. In order to begin revising species delimitation and clarifying the evolution and biology of the genus, we present a phylogenetic hypothesis using sequence data from five plastid loci (*rpoC1*, *matK* gene and flanking *trnK* intron, *atpB-rbcL* intergenic spacer, and the 3' portion of *ycf1*) and the nuclear ribosomal internal and external transcribed spacers (ITS, ETS). Resulting trees using both Bayesian and parsimony inference are congruent with each other, and generally well resolved. Based on current level of sampling across Maxillariinae, these molecular data support the monophyly of *Mormolyca* and shed light on the interspecific phylogenetic patterns within the genus. These include an early divergent paraphyletic grade of *Mormolyca* species successively sister to a clade with at least two definable subclades within. The latter are characterized by two different flower morphologies that are likely related to their pollination systems. Although not all relationships within the genus are fully resolved or supported, these results offer a first glimpse into the phylogeny of a small group of epiphytic orchids characterized by an unusually high level of variable vegetative characters, floral fragrance profiles, and pollination systems.

**KEY WORDS:** Maxillariinae, *Mormolyca*, molecular phylogenetics, Bayesian inference, Orchidaceae onomy

## Introduction

The orchid subtribe Maxillariinae (subfamily Epidendroideae: tribe Cymbidiaceae) is one of the most conspicuous and vegetatively diverse groups of Neotropical orchids (Dressler 1993; Whitten 2009). As a result, reconstructing evolutionary relationships among its more than 750 species historically has been challenging (Christenson 2002). However, molecular phylogenetic analyses published by Whitten *et al.* (2007) using DNA sequences from more than 600 specimens allowed for a new interpretation of the subtribe that has been useful for redefining particular genera (Whitten & Blanco 2011). Based on well-supported clades in the gene trees and defined by morphological synapomorphies, several genera of Maxillariinae were recircumscribed by Blanco *et al.* (2007). One genus in particular, *Mormolyca* Fenzl, changed significantly. Molecular phylogenetic analyses retrieved a strongly supported clade that included a paraphyletic *Mormolyca* s.s. sister to a clade composed of species from the previously recognized *Maxillaria*

*rufescens* complex, with the genus *Chrysocycnis* embedded within it (Whitten *et al.* 2007). These now have been transferred into *Mormolyca* to achieve monophyly of the genus, thereby expanding it from six to ca. 27 species, and increasing the range of floral and vegetative diversity within the group. *Mormolyca*, therefore, represents another example of the way in which traditional orchid classification systems that have relied almost entirely on floral morphology do not always accurately reflect the evolutionary history of their taxa.

The genus *Mormolyca* as originally circumscribed by Garay and Wirth (1959) was differentiated from *Maxillaria* on the basis of morphological characters such as its long inflorescence, absence of a column foot, and moon-shaped viscidium. Species of the *Maxillaria rufescens* complex (a.k.a. the *Rufescens* complex) are vegetatively similar to *Mormolyca* s.s. in their shortly creeping rhizomes with unifoliate pseudobulbs subtended by papery bracts (Carnevali Fernández-Concha *et al.* 2001), but the inflorescences

are much shorter. On the opposite side of the spectrum, the two species previously placed in *Chrysocycnis* are characterized by their elongate, erect rhizomes between scattered unifoliate pseudobulbs (Sweet 1971).

Plants from both *Mormolyca* s.s. and the former concept of *Chrysocycnis* have flat open flowers with a tomentose, insect-like labellum and arcuate column (especially pronounced in *Chrysocycnis*). Given that flowers of *M. ringens* are known to be pollinated by male bees through a syndrome of deceit pseudocopulation (Singer *et al.* 2004), we expect many or all species of *Mormolyca* with similar insectiform flowers (Fig. 1 G, H, J & M), to be pollinated by sexual deceit—a pollination system that was made famous over the past century by studies of unrelated terrestrial orchids in Europe, Australia, and South Africa (Stoutamire 1974, 1983; Paulus & Gack 1990; Schiestl *et al.* 2003; Johnson & Morita 2006; van der Niet *et al.* 2011). In contrast, some of the *Mormolyca* species transferred from the *Rufescens* complex have semi-open flowers with a labellum pad of short, glandular trichomes (Fig. 1 A-C, E, K, L, N, O, Q, R), and exhibit a conspicuous diversity of pleasant floral scents (Christenson 2002; Flach *et al.* 2004; pers. obs.). These orchids are almost certainly not sexually deceptive but instead appear to offer rewards to their pollinators in the form of specialized, nutrient-rich trichomes (Davies *et al.* 2000; Davies & Turner 2004; Davies & Stpiczyńska 2012). The remainder of the species from the former *Rufescens* complex have either resin-secreting or resin-mimic flowers (Davies *et al.* 2012) with glossy labella (Fig. 1. D, F, I, P), and a faint (sometimes absent), sweet, floral scent (personal obs.). These particular examples of highly specialized and varied floral forms indicate that pollinator-mediated selection probably played an important role in the diversification of *Mormolyca*. Thus, the newly expanded concept of the genus presents an especially appealing group to examine in greater detail from the perspective of taxonomy, systematics, and evolution.

The relationship between *Mormolyca* s.s. and the *Rufescens* complex was initially suggested by analyses of anatomical and morphological characters (Atwood & Mora de Retana 1999; Holtzmeier *et al.* 1998), and subsequently confirmed by phylogenetic analyses of molecular data (Dathe & Dietrich 2006; Whitten *et al.* 2007). However, our knowledge of the evolutionary relationships within the group is still quite limited, as

more species need to be incorporated into analyses. These include a handful of recently discovered species (Bogarín & Pupulin 2010; R. Arevalo and G. Carnevali unpubl. data). Not only is the genus now defined by vegetative rather than floral synapomorphies, but chemical characters related to pollination systems may also be useful for clarifying species boundaries. To understand patterns of diversification within the genus and in order to revise species delimitations that will ultimately lead to a stable classification of these orchids, a thoroughly sampled and well-supported phylogenetic framework is required. In this study we increase the dimensions of previously published molecular data sets by increasing taxon and gene samplings. We also implement alternative methods of molecular phylogenetic analysis (maximum parsimony and Bayesian inference) in an attempt to reconstruct a fully resolved and highly supported molecular phylogeny of *Mormolyca* that can be used in subsequent evolutionary studies and taxonomic revision of the genus.

### Materials and methods

*Taxon sampling* — From the ca. 27 species estimated to be in the new broad concept of *Mormolyca* (Blanco *et al.* 2007), we sampled 23 species/morphospecies (Table 1). In addition to the 17 used by Whitten *et al.* (2007), we increased the number of samples and species by targeting missing taxa from areas poorly represented in the original matrices, namely Colombia, Ecuador, and Peru. Our outgroup taxa comprise 36 species from 16 other genera of Maxillariinae (Whitten *et al.* 2007), including three recently described species new to science (Arévalo *et al.* 2013). A few specimens could not be identified unequivocally to species; they may represent new species or are elements of highly variable species complexes. These are identified with either the species modifier “c.f.” or the name of its putative closest relative or with the abbreviation “sp. nov.” after the genus, respectively. Even though our sampling of *Mormolyca* s.l. is still 26% incomplete, we are confident that we have included enough representatives of the genus to begin making assertions about the evolutionary relationships among the taxa.

*DNA extraction, amplification, and sequencing* — Once specimens were obtained, plant tissue was



TABLE 1. Voucher information for taxa used in this study. Herbarium acronyms: COL = Universidad Nacional de Colombia, Bogotá D.C., Colombia; CR = Museo Nacional de Costa Rica, San José, Costa Rica; FLAS = University of Florida; JBL = Jardín Botánico Lankester, Universidad de Costa Rica, Cartago, Costa Rica; M = Botanische Staatssammlung München, München, Germany; SEL = Marie Selby Botanical Gardens, Sarasota, FL, U.S.A.; QCA = Pontificia Universidad Católica del Ecuador, Quito, Ecuador; SP = Instituto de Botânica, São Paulo, Brazil; UEC = Universidade Estadual de Campinas, Brazil; WIS = University of Wisconsin, Madison, U.S.A. Figure 3. Majority-rule consensus of 7500 trees obtained from Bayesian analysis (GTR + gamma model of evolution) of combined plastid and nuclear DNA regions. Numbers above branches are bootstrap percentages; numbers below branches are Bayesian posterior probabilities. Colored branches indicate the groups discussed in the text. Letter codes following the taxon name, where present, represent the country of provenance: CLM=Colombia, COS=Costa Rica, ECU=Ecuador, HON=Honduras, PAN=Panama, PER=Peru, PUE=Puerto Rico.

	Taxon	Collector and number	Source	Herbarium
1	<i>Brasiliorchis picta</i> (Hook.) R.B.Singer, S.Koehler & Carnevali	Whitten 2755	Brazil, cult.	FLAS
2	<i>Brasiliorchis schunkeana</i> (Campacci & Kautsky) R.B.Singer, S.Koehler & Carnevali	Whitten 1992	Brazil, cult.	FLAS
3	<i>Bifrenaria tetragona</i> (Lindl.) Schltr.	Whitten 93156	Brazil	FLAS
4	<i>Camardium carinulatum</i> (Rchb.f.) M.A.Blanco	Arévalo 932	Colombia	COL
5	<i>Camardium ochroleucum</i> Lindl.	Gerlach 2003–3648	Brazil	M
6	<i>Christensonella ferdinandiana</i> (Barb.Rodr.) Szlach., Mytnik, Górnjak & Smiszek	Koehler 0089	Brazil	SP
7	<i>Christensonella nardoides</i> (Kraenzl.) Szlach.	Whitten 2502	Ecuador, cult. Ecuagenera	FLAS
8	<i>Cryptocentrum peruvianum</i> (Cogn.) C.Schweinf.	Whitten 2322	Ecuador	FLAS
9	<i>Cryptocentrum</i> sp.	Arévalo 931	Colombia	COL
10	<i>Cyrtidiorchis alata</i> (Ruiz & Pav.) Rauschert	Whitten 2932	Ecuador, cult.	FLAS
11	<i>Cyrtidiorchis rhomboglossa</i> (F.Lehm. & Kraenzl.) Rauschert	Giraldo 17	Colombia	COL
12	<i>Eriopsis biloba</i> Lindl.	Whitten 3153	Ecuador	QCA
13	<i>Heterotaxis villosa</i> (Barb.Rodr.) F.Barros	Arévalo 902	Colombia	COL
14	<i>Heterotaxis violaceopunctata</i> (Rchb.f.) F.Barros	Whitten 2294	Brazil, cult.	FLAS
15	<i>Inti bicallosa</i> (Rchb.f.) M.A.Blanco	Whitten 2636,	Panama	FLAS
16	<i>Inti chartacifolia</i> (Ames & C.Schweinf.) M.A.Blanco	Whitten 2752	cult.	FLAS
17	<i>Mapinguari auyantepuiensis</i> (Foldats) Carnevali & R.B.Singer	Whitten 2347	Ecuador	FLAS
18	<i>Mapinguari longipetiolatus</i> (Ames & C.Schweinf.) Carnevali & R.B.Singer	Atwood & Whitten 5075	Costa Rica	SEL
19	<i>Maxillaria farinosa</i> Arévalo & Christenson, sp. nov.	Arévalo 734	Colombia	COL
20	<i>Maxillaria splendens</i> Poepp. & Endl.	Koehler 0144	Brazil, cult.	UEC
21	<i>Maxillaria tenebrifolia</i> Arévalo & Christenson, sp. nov.	Arévalo 454	Colombia	COL
22	<i>Maxillariella procurrens</i> (Lindl.) M.A.Blanco & Carnevali	Whitten 2397	Ecuador, cult.	FLAS
23	<i>Mormolyca</i> cf. <i>acutifolia</i> (Lindl.) M.A.Blanco	1: Arévalo 1071	Colombia, cult. Colomborquideas	WIS
		2: Giraldo 44	Colombia	COL
24	<i>Mormolyca</i> cf. <i>aureoglobula</i> (Christenson) M.A.Blanco	Arévalo 1069	Colombia, cult. Orquídeas del Valle	WIS
25	<i>Mormolyca chacoensis</i> (Dodson) M.A.Blanco	Arévalo 947	Perú, cult., Agroriente Viveros	COL

TABLE I. *Continued.*

26	<i>Mormolyca culebrica</i> Bogarín & Pupulin	<i>Whitten 2650</i>	Pánama, cult.	FLAS
27	<i>Mormolyca dressleriana</i> (Carnevali & J.T.Atwood) M.A.Blanco	1: <i>Arévalo 1066</i>	Pánama, cult.	WIS
		2: <i>Arévalo 1065</i>	Costa Rica, cult.	WIS
28	<i>Mormolyca fumea</i> Bogarín & Pupulin	<i>Bogarín 5729</i>	Costa Rica	CR
29	<i>Mormolyca gracilipes</i> (Schltr.) Garay & Wirth	<i>Arévalo 1061</i>	Colombia, cult., Orquídeas del Valle	WIS
30	<i>Mormolyca hedwigiae</i> (Hamer & Dodson) M.A.Blanco	1: <i>Koehler 0314</i>	Guatemala, cult.	ESA
		2: <i>Arévalo 1065</i>	Honduras, cult.	WIS
31	<i>Mormolyca moralesii</i> (Carnevali & J.T.Atwood) M.A.Blanco	1: <i>Bogarín 3826</i>	Costa Rica	JBL
		2: <i>Bogarín 4139</i>	Costa Rica	JBL
32	<i>Mormolyca peruviana</i> C.Schweinf.	<i>Whitten 2497</i>	Ecuador, cult.	FLAS
33	<i>Mormolyca polyphylla</i> Garay & Wirth	<i>Arévalo 950</i>	Ecuador, cult. Ecuagenera	COL
34	<i>Mormolyca pudica</i> (Carnevali & J.L.Tapia) M.A.Blanco	<i>Arévalo 1068</i>	Puerto Rico	WIS
35	<i>Mormolyca richii</i> (Dodson) M.A.Blanco	1: <i>Whitten 2362</i>	Ecuador, cult.	FLAS
		2: <i>Arévalo 1064</i>	Ecuador, cult.	WIS
36	<i>Mormolyca ringens</i> (Lindl.) Gentil	<i>Arévalo 1062</i>	Colombia, cult. Orquídeas del Valle	WIS
37	<i>Mormolyca rufescens</i> (Lindl.) M.A.Blanco	1: <i>Arévalo 1073</i>	U.S.A., cult. Marie Selby Botanical Gardens	WIS
		2: <i>Arévalo 941</i>	Perú, cult., Agroriente Viveros	WIS
		3: <i>Arévalo 1076</i>	U.S.A., cult. Marie Selby Botanical Gardens	WIS
		4: <i>Arévalo 1075</i>	U.S.A., cult. Marie Selby Botanical Gardens	WIS
		5: <i>Arévalo 942</i>	Perú, cult., Agroriente Viveros	COL
		6: <i>Arévalo 943</i>	Perú, cult., Agroriente Viveros	COL
38	<i>Mormolyca sanantonioensis</i> (Christenson) M.A.Blanco	<i>Arévalo 1070</i>	Colombia, cult. Orquídeas del Valle	WIS
39	<i>Mormolyca schlimii</i> (Linden & Rchb.f.) M.A.Blanco	<i>Giraldo 763</i>	Colombia	COL
40	<i>Mormolyca schweinfurthiana</i> Garay & Wirth	<i>Arévalo 956</i>	Ecuador, cult. Ecuagenera	COL
41	<i>Mormolyca suareziorum</i> (Dodson) M.A.Blanco	<i>Arévalo 945</i>	Perú, cult., Agroriente Viveros	COL
42	<i>Mormolyca</i> cf. <i>suareziorum</i> (Dodson) M.A.Blanco	<i>Whitten 2758</i>	Ecuador, cult.	FLAS
43	<i>Mormolyca</i> cf. <i>tenuibulba</i> (Christenson) M.A.Blanco	1: <i>Arévalo 878</i>	Colombia	COL
		2: <i>Arévalo 951</i>	Ecuador, cult., Ecuagenera	COL
		3: <i>Arévalo 1072</i>	Colombia, cult. Colomborquídeas	WIS
44	<i>Mormolyca</i> sp. nov. A	<i>Arévalo 1063</i>	U.S.A., cult., Marie Selby Botanical Gardens	WIS
45	<i>Mormolyca</i> sp. nov. B	<i>Blanco 3108</i>	Ecuador, cult.	FLAS
46	<i>Mormolyca</i> sp. nov. C	1: <i>Arévalo 1074</i>	Colombia, cult. Colomborquídeas	WIS
		2: <i>Arévalo 939</i>	Perú, cult., Agroriente Viveros	COL
		3: <i>Arévalo 953</i>	Perú, cult., Agroriente Viveros	COL

TABLE 1. *Continued.*

47	<i>Nitidobulbon nasutum</i> (Rchb.f.) Ojeda & Carnevali	Whitten 1869	Ecuador	FLAS
48	<i>Nitidobulbon proboscideum</i> (Rchb.f.) Ojeda & Carnevali	Atwood & Whitten 5056	Venezuela	SEL
49	<i>Ornithidium montezumae</i> Arévalo & Christenson	Arévalo 674	Colombia	COL
50	<i>Ornithidium</i> cf. <i>semiscabrum</i> Lindl.	Arévalo 588	Colombia	COL
51	<i>Ornithidium aggregatum</i> (Kunth) Rchb.f.	Arévalo 623	Colombia	COL
52	<i>Pityphyllum saragurense</i> (Dodson) Whitten	Whitten 3084	Ecuador, cult.	QCA
53	<i>Pityphyllum antioquiense</i> Schltr.	Whitten 2473	Ecuador, cult.	FLAS
54	<i>Rhetinantha acuminata</i> (Lindl.) M.A.Blanco	Whitten 2698	Ecuador	FLAS
55	<i>Rhetinantha notylioglossa</i> (Rchb.f.) M.A.Blanco	Koehler 0033	Brazil	UEC
56	<i>Sauvetrea alpestris</i> (Lindl.) Szlach.	Whitten 2551	Ecuador, cult.	FLAS
57	<i>Sauvetrea laevilabris</i> (Lindl.) M.A.Blanco	Whitten 2358	Ecuador, cult.	FLAS
58	<i>Trigonidium egertonianum</i> Bateman ex Lindl.	Arévalo 1060	cult.	WIS
59	<i>Trigonidium obtusum</i> Lindl.	Whitten 2997	cult.	FLAS

preserved in silica gel, and genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, U.S.A.). Living specimens were documented with detailed photographs prior to being pressed for herbarium vouchers. When possible, flowers were collected and preserved in FAA for micro-morphological assessment.

For each specimen we attempted to sequence the plastid *matK* gene and flanking *trnK* intron, the *atpB-rbcL* intergenic spacer, the *rpoCl* gene, and the downstream (3') portions of *ycf1*, along with the nuclear ribosomal ITS and ETS regions. DNA amplification and sequencing was carried out following published primers and methods, with modifications when necessary (*matK+trnK*, *atpB-rbcL*, *rpoCl* and ITS from Whitten *et al.* 2007; *ycf1* from Neubig *et al.* 2009; ETS from Monteiro *et al.* 2010). Electropherograms were assembled and edited using Geneious Pro 5.0.3 (Drummond *et al.* 2010); alignments were generated using MUSCLE (Edgar 2004) and adjusted by eye using MacClade (Maddison & Maddison 2005).

*Data analysis*—Phylogenetic inference methods included maximum parsimony (MP) and Bayesian inference (BI). We performed combined analysis with ITS + ETS (nrDNA), all four plastid regions (cpDNA), and finally with all regions (total DNA). MP analyses were performed in PAUP\* 4.10b (Swofford 2002) with Fitch parsimony as the optimality criterion (unordered characters, equal weights; Fitch 1971),

ACCTRAN optimization, and gaps treated as missing data. The heuristic search strategy consisted of 5000 random-addition replicates of branch swapping by subtree-pruning-regrafting (SPR), saving multiple trees (MULTREES), and holding five trees at each step. The resulting trees were then used as starting trees for tree-bisection-reconnection swapping (TBR). Levels of support were estimated from 1000 bootstrap replicates, using TBR swapping for five random-addition replicates per bootstrap replicate. Parsimony ratchet search strategy for finding shortest trees was also performed with the program PAUPRat (Sikes & Lewis 2001). The software package MRBAYES v3.2.1 (Ronquist *et al.* 2012) was used for BI analyses. Tree searches were performed assuming single and multiple models of sequence evolution for each partition, following the “Akaike information criterion” as implemented in JModeltest (Posada 2008). For each analysis, Markov chain Monte Carlo (MCMC) searches were made for 10 million generations, sampling every 1000 generations, with a burn-in of 25% and chains heated to 0.07 (increasing the frequency of data swapping between chains).

## Results

Statistics associated with MP analyses of cpDNA, nrDNA, and total DNA data sets are summarized in Table 2. Analysis of both cpDNA and nrDNA data sets strongly support the monophyly of *Mormolyca*,

TABLE 2. Features of DNA data sets used in this study. CI = consistency index; RI = retention index. Percentages calculated in relation to aligned length.

Data set	No. of ingroup taxa	Aligned length	No. of variable characters	No. of parsimony-informative characters	No. of most parsimonious trees	Tree length	CI	RI
nrDNA	38	1368	546 (39.9%)	301 (22%)	126,660	1129	0.637	0.716
cpDNA	38	7242	1254 (17.3%)	521 (7.2%)	88,329	2155	0.650	0.706
totalDNA	38	8610	1801 (20.9%)	822 (9.5%)	8353	3373	0.629	0.688

although relationships within the genus differ in the absence of strongly supported clades in the nrDNA analysis (trees not shown). By comparison, the total DNA matrix offered the highest level of resolution among all genera and within *Mormolyca*, as assessed by bootstrap support. The strict consensus tree from MP analysis of the total DNA data set is presented in Fig. 2. For BI analysis, the model-based estimate based on the cpDNA regions produced a tree with higher levels of support when compared to the nrDNA tree (trees not shown). Consensus trees from BI and MP were similar, but the Bayesian tree was more highly resolved within *Mormolyca*. This is the tree upon which our discussion will follow (Fig. 3).

Placement of the recently described species (*Maxillaria farinosa*, *M. tenebrifolia*, and *Ornithidium montezumae*) within their respective lineage in the overall tree confirm their assigned genera (Fig. 2). *Mormolyca* s.l. forms a monophyletic group with strong support. Taxa within the genus can be divided into three clusters (showed with color branches on tree) for the sake of further discussion: a grade of early divergent species and two clades (Fig. 2).

*Mormolyca* s.s. species (green branches on tree) are the earliest extant lineages to diverge from the genus, represented here by *M. polyphylla*, *M. peruviana*, *M. schweinfurthiana*, *M. gracilipes*, and *M. ringens*, ending with *M. schlimii*, which is sister to the single large clade containing all members of the former *Rufescens* complex (Fig. 2). Topographically, this clade, consists of clade I (purple branches) with weak support (BP 69%, PP 1.0) but fully resolved, and clade II (orange branches) that is not fully resolved.

## Discussion

This study confirms the inclusion of species from the *Maxillaria rufescens* complex and *Chrysocynis* species within *Mormolyca* (Blanco *et al.* 2007, Whitten

*et al.* 2012). In general, as now defined, *Mormolyca* s.l. can be recognized by the following combination of characters: unifoliate pseudobulbs subtended by a non-foliaceous (papery) sheaths (except for *M. polyphylla*, which has elongated pseudobulbs subtended by more than one sheath and up to three apical leaves), the single-flowered, erect inflorescence produced from the older parts of the rhizome (rather than from the terminal growth), perianth parts that lack fibers, and the clavate, arcuate column (Blanco *et al.* 2007). Distribution of the genus ranges from southern Mexico in the north to Bolivia and northern Brazil in the south, with plants typically found in forests at elevations from sea level to 1900 m.a.s.l. (based on gathered data from herbarium specimens). One taxon, *M. pudica*, is apparently restricted to the Greater Antilles (Carnevali Fernandez-Concha *et al.* 2001). A weak geographic pattern can be detected in the phylogenetic reconstruction, with most of the early-diverging species restricted to the southern part of the range, (i.e., from Peru, Ecuador, and Colombia), whereas the more derived clades have a more northern distribution, reaching all the way to southern Mexico.

The consecutively early-diverging and paraphyletic taxa are represented here by most species of *Mormolyca* s.s. and *M. schlimii*. All are morphologically similar, particularly in their flowers (i.e. absence of a column foot, the insectiform labellum, and the prominent arcuate column). Unfortunately, our study did not include *M. aurorae* or *M. fuchsii*, which are known only from the type specimens, and *M. lehmanii*, for which we were unable to obtain samples. We would expect these species to be positioned in this part of the tree as well. The appearance of *M. gracilipes* and *M. schweinfurthiana* as sister species may reflect a misidentification of one of the samples and/or poor alpha-taxonomy. These two species are similar in form, and the two names are often incorrectly applied. We



FIGURE 1. A. *Mormolyca acutifolia*; B. *M. aureoglobula*; C. *M. cf. chacoensis*; D. *M. culebrica*; E. *M. dressleriana*; F. *M. hedwigiae*; G. *M. peruviana*; H. *M. polyphylla*; I. *M. richii*; J. *M. ringens*; K. *M. rufescens*; L. *M. sanantonioensis*; M. *M. schweinfurthiana*; N. *M. suareziyorum*; O. *M. tenuibulba*; P. *M. sp. nov. A*; Q. *M. sp. nov. B*; R. *M. sp. nov. C*. All photos by R. Arévalo, except J & M by D. Bogarin.

suspect that they may, in fact, be describing the same species. However, more material including the type material needs to be examined to make a conclusive decision regarding their taxonomy.

Looking at the species distribution within the larger clade that groups the entire *Rufescens* complex,

patterns seem to emerge. Although there is only weak bootstrap support (BS 69%, PP 1.0) for this group, species with a glossy labellum cluster together. Some of these secrete small quantities of resin on the labellum and are thought to mimic taxa that produce lipoidal rewards (Davies *et al.* 2012; Arévalo, unpubl. data). This clade includes one of the new species that was found as a result of this study (*Mormolyca* sp. nov. A), which is sister to the rest of the clade consisting of *M. richii*, *M. hedwigiae*, *M. culebrica*, and *M. fumea*. The latter, which is not recognized by the World Checklist of Selected Plant Families (Govaerts *et al.* 2011) and considered a synonym of *M. aureoglobula*, appears as a distinct branch sister to *M. culebrica* in our analyses. This result indicates that it probably does deserve to be treated as a distinct species.

The remaining *Mormolyca* species from the former *Rufescens* complex, characterized by their semi-open, fragrant flowers with a labellum pad of short, glandular trichomes (Flach *et al.* 2004; pers. obs.), also cluster together. Species delimitation in this group has been difficult historically, with nearly every species of the complex included at one point either in a broad concept of either *Mormolyca rufescens* (Lindl.) Blanco, or *M. acutifolia* (Lindl.) Blanco. Large-flowered specimens with conspicuous fragrances are usually associated with *M. rufescens*, whereas all small-flowered entities are usually considered to be related to *M. acutifolia*. A factor contributing to this taxonomic confusion is the fact that *M. acutifolia* (Lindl.) Blanco is poorly defined. Other than the type specimen itself, there is only a vague description from Lindley (1839) and a single drawing of the flower labellum found with the holotype.

Also, within this cluster we find *M. dressleriana* as sister to a group of species in which relationships and alpha-taxonomy are still imprecise. This group reunites what we are considering *M. acutifolia* along with some entities originally related to the species and now segregated (*i.e.* *M. pudica*, *M. aureoglobula*, *M. sanantonioensis*, *M. moralesii*). It also contains multiple individuals of *M. tenuibulba*, a species characterized by having ascending rhizomes with long pseudobulbs and coconut-scented flowers. Accessions of this species are not monophyletic, which we believe may be due to incomplete data sampling. A new morphologically distinct species



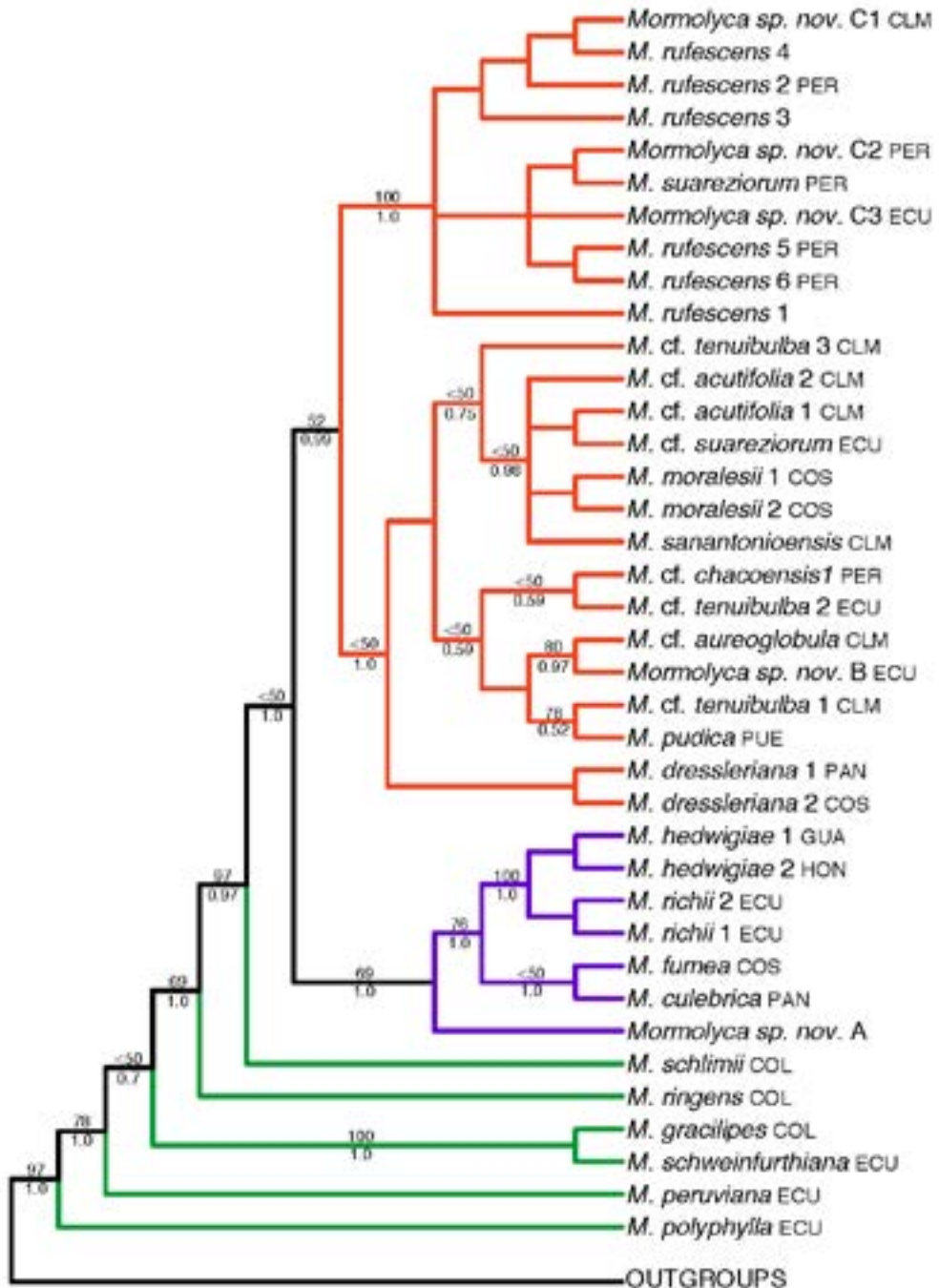


FIGURE 3. Majority-rule consensus of 7500 trees obtained from Bayesian analysis (GTR + gamma model of evolution) of combined plastid and nuclear DNA regions. Numbers above branches are bootstrap percentages; numbers below branches are Bayesian posterior probabilities. Colored branches indicate the groups discussed in the text. Letter codes following the taxon name, where present, represent the country of provenance: CLM=Colombia, COS=Costa Rica, ECU=Ecuador, HON=Honduras, PAN=Panama, PER=Peru, PUE=Puerto Rico.

of *Mormolyca* previously recognized (G. Carnevali, pers. comm. 2008), also appears here (*Mormolyca* sp. nov. B). In general, the topology recovered for this part of the tree indicates that species boundaries need to be better defined in this group. A revision is currently in progress.

Finally, we recovered a well-supported clade that includes all large-flowered plants associated with *M. rufescens*, as well as the small-flowered species *M. suareziorum*, along with accessions of what we are considering another new species of *Mormolyca* (*Mormolyca* sp. nov. C) from three different locations. We included multiple samples of *M. rufescens* that vary in flower color pattern, a character often used to segregate new species of *Mormolyca* (e.g. Christenson 2010), to test taxonomic hypotheses of reciprocal monophyly. Unfortunately, precise relationships among these accessions are unresolved in our analyses, and so issues of species delimitations will require further study.

In summary, despite our efforts to resolve phylogenetic relationships more fully within *Mormolyca*, more work still remains to be done. Species delimitation in this genus is difficult, but we feel strongly that variation in flower color should be reconsidered or even disregarded as a character used to segregate species. Given the importance of flower micro-morphology in their pollination systems, detailed morphological analyses are currently underway in the search for unambiguous synapomorphies. This is our first attempt to reconstruct a phylogeny of *Mormolyca*, and we are confident that the addition of genetic data from more variable loci such as the low-copy nuclear gene *PhyC* (Russell *et al.* 2010), as well as micro-morphological characters will help us achieve a better estimate of relationships within the genus. Evolutionary processes that underlie the patterns of variation and specialization exhibited by this group of plants remain to be investigated. To address these challenges and utilize *Mormolyca* as a model system for understanding orchid pollinator evolution within Neotropical epiphytic Orchidaceae, we expect eventually to couple these molecular phylogenetic data with floral morphology and patterns of volatile production to assess the role of evolving flower form and function in the process of orchid speciation.

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## ORCHIDS WITHOUT BORDERS: STUDYING THE HOTSPOT OF COSTA RICA AND PANAMA

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**ABSTRACT.** The Mesoamerican region is one of the richest in orchid diversity in the world. About 2670 species, 10% of all orchids known have been recorded there. Within this region, most of the species are concentrated in the southernmost countries. Costa Rica with 1598 species (or 0.030 spp/km<sup>2</sup>) and Panama with 1397 species (0.018 spp/km<sup>2</sup>) stand at the top of endemic species list of all Mesoamerica, with 35.37% and 28.52%, respectively. These figures, however, are misleading, as political boundaries do not have any relationship to orchid diversity. If we ignore the political frontier, there is a common biogeographic area. However, if we put the border back, the numbers in terms of scientific production and research change dramatically. Costa Rica has increased the knowledge of its orchid flora through the establishment of a successful research system, whereas Panama has lacked a similar process. To address this problem, the Lankester Botanical Garden at the Universidad de Costa Rica and the Universidad Autónoma de Chiriquí, Panama, established a new research center focused on the study of orchids. The aim of the cooperation is to provide the methodology, information, and expertise for a long-term project on taxonomy and systematics of the orchids of Panama

**KEY WORDS:** Orchidaceae, Mesoamerica, Costa Rica, Panama, floristics, taxonomy

The Mesoamerican region is one of the richest in orchid diversity in the world. About 2670 species, 10% of all orchids known, have been recorded there. The distribution of orchids in the region is not uniform. Diversity at country level depends on geological, climatic, and orographic factors as well as territorial extension. However, when examining the diversity index for Orchidaceae (calculated as number of species/km<sup>2</sup>), a trend is evident. The southern regions of the isthmus, with Costa Rica and Panama, head the list with rates of 0.03 and 0.018, respectively (Fig. 1). These numbers are also reflected in terms of absolute diversity, with 1598 and 1397 orchid species of Orchidaceae recorded for this two countries, respectively, the highest values in the region (Ossenbach *et al.* 2007; Fig. 2). Phylogenetic studies on the evolution of the family show that both countries currently have a floristic contingent of different origins, joining the rich Andean flora with the large number of taxa from nuclear Mesoamerica,

which disappear quickly in the South American flora (Fig. 3). From a phylogeographical point of view, and the peculiarity of its geological history, Costa Rica and Panama represent a laboratory for the evolution of orchids, and a critical examination of their orchid floras is of scientific priority under both the study and conservation of biodiversity (D’Arcy & Correa 1985; Dressler 2003).

Despite its geological and floristic similarities, the political boundary has affected knowledge and scientific production. A strong imbalance between the two countries is evident. In the last 10 years, about 170 scientific papers for the orchid flora of Costa Rica were published. In contrast, about 70 scientific papers were devoted to the orchids of Panama. Costa Rica has increased the knowledge of its orchid flora through the establishment of a successful research system, whereas Panama has lacked a similar process. The latest, in-depth studies on the orchids of Panama took place more

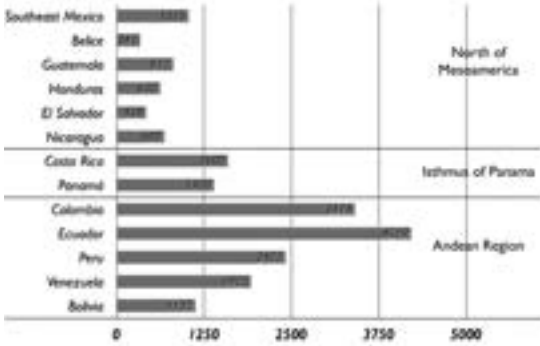


FIGURE 1. Comparison of the number of species/ km<sup>2</sup> between northern Mesoamerica, Isthmus of Panama, and the Andean countries.

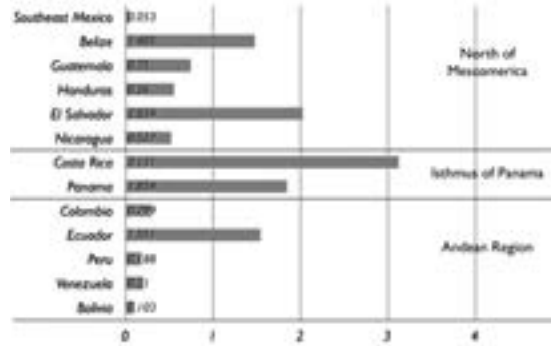


FIGURE 2. Comparison of the number of species between northern Mesoamerica, Isthmus of Panama, and the Andean countries.

than 20 years ago; recent information has been produced sporadically by researchers based in other countries (Dressler 1993; Williams & Allen 1946-1949). In this paper, we analyze the future and challenges of orchid research in the isthmus of Costa Rica and Panama. We present the main objectives of the scientific cooperation between Jardín Botánico Lankester of the Universidad de Costa Rica and the Universidad Autónoma de Chiriquí, Panamá.

**Geography**

Costa Rica and Panama are situated in the southeast of Central America (11°15'N-7°10'N, 85°56'N-77°09'W) and comprise a narrow land strip called the Isthmus of Panama that links North and South America, separating the Atlantic from the Pacific Ocean (Fig. 4). In the north of Costa Rica along the border with Nicaragua, floodplain lands separate the mountain ranges of Guanacaste from northern Central America. These chains are formed by active volcanoes such as the Rincón de la Vieja (1916 m) and Arenal (1670 m). The Cordillera Volcánica Central in Costa Rica has higher elevations with active volcanoes: Poás (2708 m), Irazú (3432 m), and Turrialba (3340 m). To the southeast lies the Cordillera de Talamanca, an intricate mountain range with the highest elevations of the isthmus, usually above 3000 m. This mountain range has no active volcanoes and is protected mostly by the La Amistad International Park. The highest peak is Cerro Chirripó (3820 m) followed by the peaks of Buenavista (3491 m), Durika (3281 m), Kamuk (3549 m), and Echandi (3162 m) in Costa Rica. Close to the border lie the highest peaks of Panama:

Volcan Barú (3475 m), Cerro Fábrega (3335 m), and Cerro Itamut (3293 m) (Fig. 5). The Cordillera de Talamanca loses elevation towards central Panama in the provinces of Veraguas and Coclé. Central Panama is the narrowest stretch of the isthmus, and elevations are relatively low. It includes Cerro Azul (571 m), Campana (1030 m), Gaital (1185 m), and Jefe (1007 m). Towards Colombia, the ridges of San Blas and Darien extend along the Pacific with the highest peak, Cerro Tacarcuna (1875 m). Along the Atlantic side, the main ranges are Sapo, Bagre, and Jurado. Other important geological formations are the peninsulas of Azuero, Burica, Osa, and Nicoya, comprising lower-elevation mountain ranges but relatively isolated from Talamanca.

**Geology**

Costa Rica and Panama act as a geological unit. Both countries lie mostly on the Costa Rica-Panama microplate, a complex area of interaction of three tectonic plates: Nazca, Cocos, and Caribe (Fig. 6). According to Coates *et al.* (1992), three major events led the tectonic evolution of the southern Central American isthmus. First is the volcanic activity favored by the convergent tectonics of the eastern Pacific subduction zone, one of the primary forces that produced the volcanic arc that extends from North America to the south. Another tectonic effect is the subduction of the Cocos Ridge on the Pacific. This submarine range led to the rise of the isthmus from the Arenal Volcano in Costa Rica to nearby Cerro Campana and Gaital at El Valle, Panama. From this region, the Cordillera de Talamanca emerged and

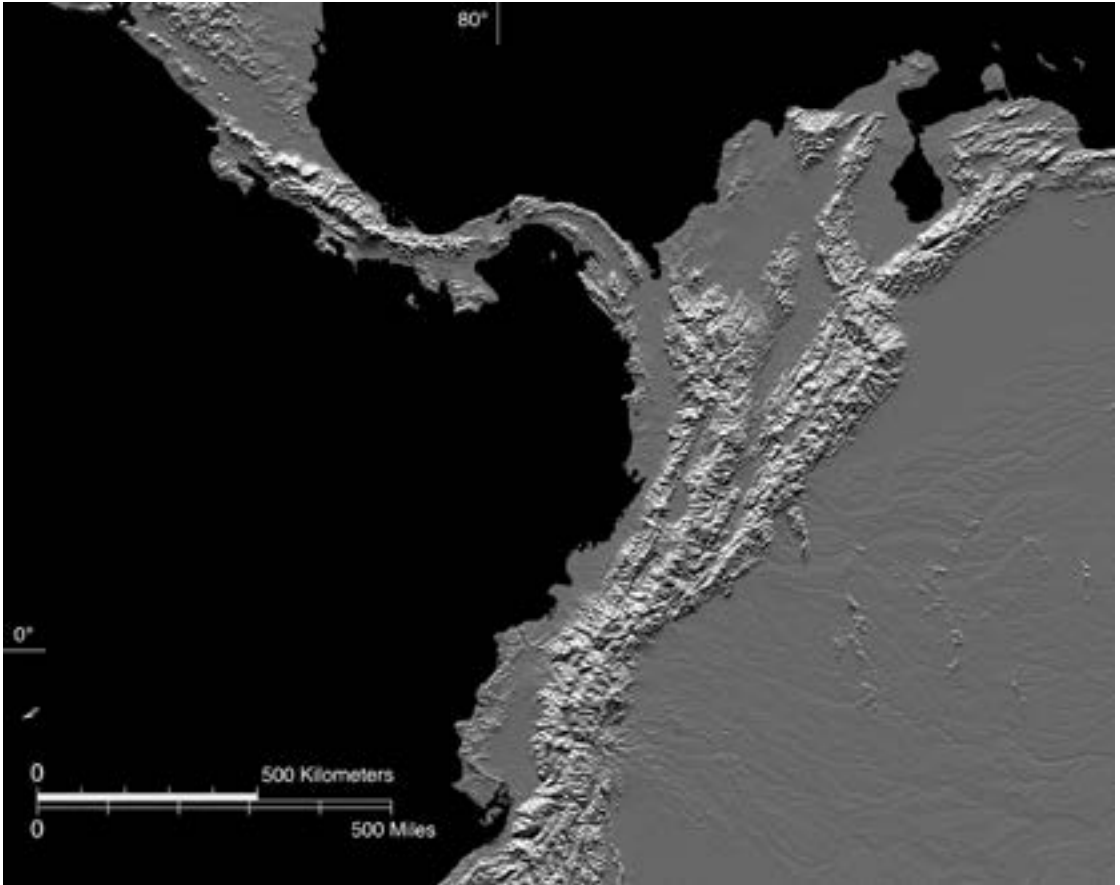


FIGURE 3. Costa Rica and Panama lie close to the Equator between North and South America.

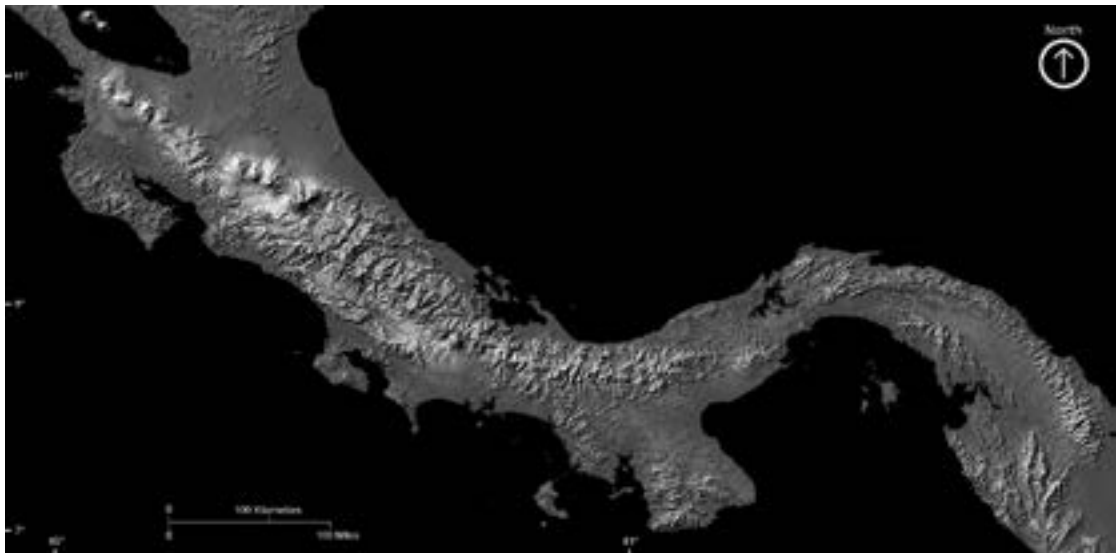


FIGURE 4. Satellite view of Costa Rica and Panama.



FIGURE 5. View of Durika-Utyum peaks at Cordillera de Talamanca in Costa Rica. Clouds are formed by the humid trade winds from the Caribbean. The Pacific side remains relatively clear. Courtesy of Daniel Jiménez.

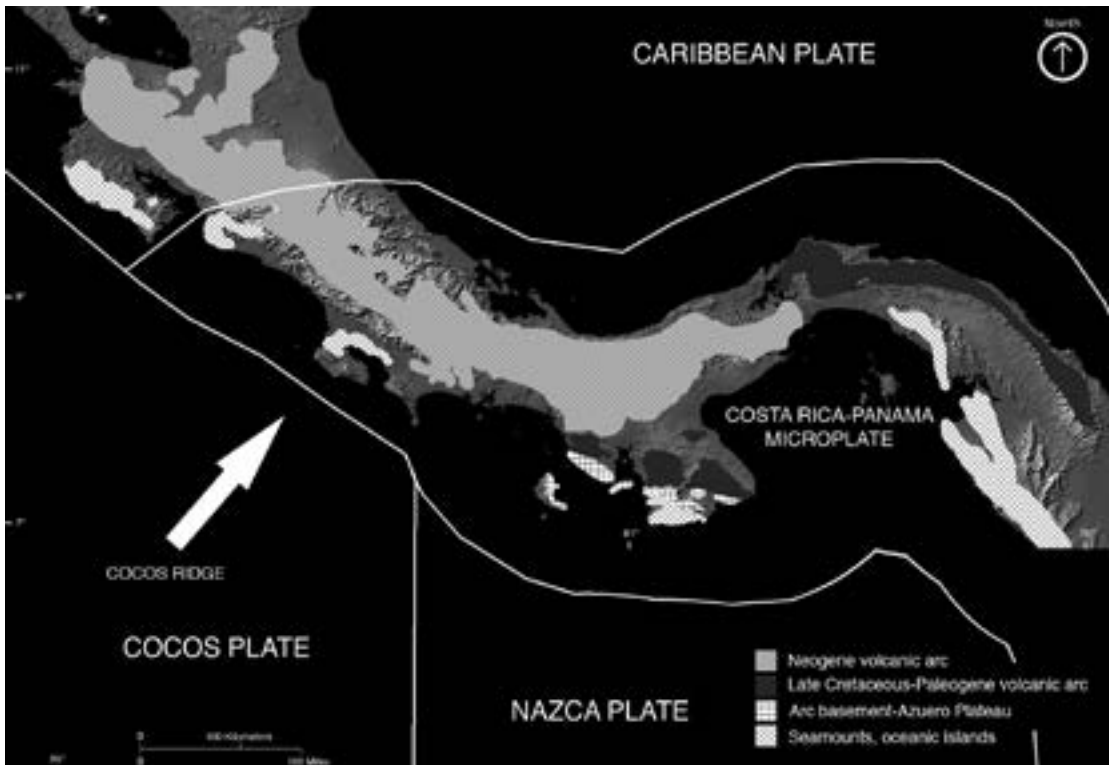


FIGURE 6. Plate tectonic configuration of the Isthmus of Panama. Both countries lie in a complex area of interaction of three tectonic plates: Nazca, Cocos, and Caribe.



FIGURE 7. Vegetation units of Costa Rica and Panama.

reduced the marine connections. Finally, the southern region of the isthmus was affected by the collision of the volcanic arc with northwestern South America and led the uplift of eastern Panama (San Blas and Darién ranges) and northern Andes of Colombia and Venezuela in the late Neogene. In general terms, the closure of the Isthmus of Panama started some 15 Myr ago in the middle Miocene and was finally closed 3.5 Myr in the Pliocene (Fig. 6).

#### Climate and vegetation

The climate and vegetation of Costa Rica and Panama are affected by their geographical position close to the Equator and surrounded by two oceans, mountain chains with different elevational ranges, and the prevailing wet winds of the Caribbean. Usually the Caribbean lowlands are moist and warm (Fig. 7). Precipitation originates mostly from the trade winds from the Caribbean colliding with the mountain ranges, mostly on the Cordillera de Talamanca and the action of the Intertropical Convergence Zone (Kohlmann *et al.* 2002). Towards the region of central Panama, there are low hills such as Campana and Gaital with montane forests fed by interaction of the wet winds. The Cordillera de Talamanca has broad elevational ranges, and the wind system is complex. The climate varies from humid in the Caribbean watershed to dry and cold at the top of the highest mountains of Talamanca. The Pacific slope of Talamanca is wet from the collision of local winds with the moist air

from the Pacific Ocean. The north Pacific region of Costa Rica and eastern Peninsula de Azuero in Panama are warm and dry with marked seasonality. To the east of Panama the climate is warm and rainy, similar to that of Chocó in Colombia, one of the wettest regions on Earth (Dressler 1993).

#### The orchid flora of Costa Rica and Panama

In general, the orchid flora of Costa Rica and Panama is a reflection of its geological history. Some factors that determine its floristic composition are the location between the great continental floras of north and south, climate, and proximity to the Andes, the most biodiverse area in the Neotropics. The lifting of the Cordillera de Talamanca with elevations up to 4000 m produced new environments for colonizing plant species from the Andean region. Recent studies on the evolution of Orchidaceae (Conran *et al.* 2009; Ramírez *et al.* 2007) support the hypothesis of an ancient origin of the family (76-84 Myr ago). Therefore, during the formation of the Isthmus that began about 15 Myr ago with the emergence of an archipelago, orchids could colonize the islands long before its closure. Some of the endemic species could also be explained by allopatric speciation produced in those ancient islands (Burger 1980). With the closure of the isthmus about 3.5 Myr and the union of the peninsulas of Nicoya, Osa, Burica, and Azuero, other species managed to colonize most of the regions of the isthmus (Fig. 6). These events may be supported by current species



FIGURE 8. Some of the endemic species of *Lepanthes* from Costa Rica and Panama.

composition. For example, most of the genera in Pleurothallidinae (the most diverse subtribe of the Neotropics) from middle and upper elevations (1500-4000 m) such as *Brachionidium* Lindl., *Lepanthes* Sw., *Pleurothallis* R.Br. s.l., and *Stelis* Sw. s.l. are more diverse in Costa Rica and Panama than in other areas of Central America. *Fernandezia* Lindl., *Telipogon* Kunth, and other terrestrial genera such as *Aa* Rchb.f. and *Petrichis* Lindl. are other examples (Dodson 2003; García Castro *et al.* 1995). However, the northern region of Costa Rica and central-southeast Panama have floras with few similarities. The northern region

of Costa Rica has a northern influence, whereas the southeast of Panama is closely related to the flora of Colombia and Ecuador. An exemplary genus to study the evolutionary floristic relationships in the isthmus is *Lepanthes* Sw. Most of the species are found in montane and premontane rain forests at elevations of 1500-2500 m. Many species are endemic to certain ridges or hills, and their distribution is often narrow. In Costa Rica there are about 120 species (Bogarín *et al.* 2012), whereas in Panama 60 species were recorded (Pupulin *et al.* 2009). Both countries share 35 species, all distributed from central and southern Costa Rica



to western Panama. In the region of central Panama, few species are shared (Fig. 8). As an example, *L. schizocardia* Luer, described from the region of Cerro Jefe, was reported for Costa Rica (Dressler 2003). However, a study of the populations from Costa Rica and Panama showed that *L. schizocardia* is an endemic of central Panama, sister of two endemic species from Costa Rica, *L. montis-narae* Pupulin, Bogarín & C.M. Sm. and *L. sotoana* Pupulin, Bogarín & C.M. Sm., both formerly treated as *L. schizocardia* (Pupulin *et al.* 2010). With the advancement of floristic research in both countries we expect to present a more detailed picture of their complex floristic relationships.

### A review of the history of the orchidology in Costa Rica and Panama

The botanical exploration of Costa Rica and Panama, which mainly began in the nineteenth century with the visits of E. Arce, G. Barclay and R.B. Hinds, H. Cuming, A.S. Oersted, G. Skinner, J. von Warszewicz, and H. Wendland, led to the discovery of the first orchid species in the region (Ossenbach 2009; Fig. 9). As a side effect of the search for a possible route for the construction of a canal, exploration continued in the nineteenth and early twentieth centuries thanks to a large group of resident naturalists. Efforts by A. Endrés and later H. Pitter, A. Tonduz, A. Brenes, and C. Wercklé among others contributed greatly to reveal the rich flora of the isthmus. Within this group of naturalists, who through their work made Costa Rica the best-known country in Mesoamerica in terms of its orchid diversity profile, was the Charles H. Lankester (Fig. 10). His collection of Orchidaceae, gathered in a period of almost 40 years in the environs of Cartago, the old capital of Costa Rica, was an obligatory stop for botanists who visited Costa Rica and provided countless new species to science. Most of the species were described by Oakes Ames of Harvard and his colleagues over more than three decades of intensive floristic work (Fig. 11). The legacy of C. H. Lankester formed the basis for the creation of the Jardín Botánico Lankester (JBL) in 1973 as part of the Universidad de Costa Rica (UCR) (Fig. 12). With the creation of JBL, Costa Rica secured a permanent institution devoted to the study of orchids. Meanwhile, after the publication of *Orchidaceae Powellianae Panamenses* by Schlechter (1922) and *Flora of Barro Colorado Island* by Standley (1927), Panama received



FIGURE 9. **A.** Hugh Cuming (1791-1865). From a lithograph by Hawkins, 1850. Courtesy of Rudolf Jenny. **B.** Josef Ritter von Rawiez Warszewicz (1812-1866). Courtesy of the Hunt Institute for Botanical Documentation.



FIGURE 10. Charles H. Lankester in 1936. Courtesy of Ricardo Lankester.

a strong impetus from L. O. Williams and P. Allen who published the *Flora of Panama: Orchidaceae* (Williams & Allen 1946-1949), the first formal treatment of the country's flora (Fig. 13).

Since 1963 and for over 20 years, R.L. Dressler has undertaken the systematic study of orchids of Panama,



FIGURE 11. Oakes Ames (1874-1950). Courtesy of the Oakes Ames Herbarium, Harvard University.



FIGURE 14. Robert L. Dressler looking for orchids in Ecuador, 2007.



FIGURE 12. Inauguration of the Lankester Botanical Garden on March 2, 1973. Courtesy of Ricardo Lankester.



FIGURE 13. A. Louis O. Williams (1908-1991). B. Paul H. Allen (1911-1963). Courtesy of L. D. Gómez.

culminating in the publication of the *Field Guide to the Orchids of Costa Rica and Panama* (Dressler 1980, 1993; Fig. 14). After his retirement from the Smithsonian Tropical Research Institute in 1986, orchid research in Panama was conducted sporadically by visiting researchers without a permanent ongoing plan

of exploration and research (Correa *et al.* 2004). The absence of an active permanent center similar to JBL in Costa Rica slowed the spread of knowledge in Panama. Again, a clear example is *Lepanthes*. In *Flora of Panama*, Williams & Allen (1946-1949) recorded eight species. Later, Dressler (1993) reported 54 species in collaboration with C.A. Luer, who sporadically visited Panama (Luer & Dressler 1986). Since 1993 the number of species did not change significantly, and only six new species were added (Luer 1999). In contrast, about 53 species were recorded from Costa Rica since 1993 to the present (Bogarín *et al.* 2012). *Lepanthes* is just an example of the differences in scientific output between the two countries due to the lack of an ongoing project for the study of Orchidaceae in Panama.

#### Jardín Botánico Lankester and scientific production in Costa Rica

Despite their great orchid diversity, tropical regions lack important centers of orchid information. Even today, they are concentrated in Europe and North America. For historical reasons, the study of

tropical plants was performed mainly by European and American botanists in the mid-nineteenth century and the late twentieth century. This produced the information, documents, and type specimens that were deposited in herbaria and libraries far from the countries of origin. To understand and protect their biodiversity, it has become imperative for tropical countries to establish centers or institutions dedicated to the study of living organisms. The AMO Herbarium in Mexico, the Rio Palenque Science Center in Ecuador, and the Jardín Botánico Lankester in Costa Rica are research centers that contributed greatly to the development of scientific projects based on their country of origin (Atwood & Mora de Retana 1999; Dodson 2004; Pupulin 2010; Hágsater *et al.* 2005). It is necessary to expand these efforts to other Neotropical countries with the aim of having an active network of institutions.

The constraints to scientific study in the tropics have been the difficulty in access to information and the lack of an appropriate system of enquiry. Scientific progress made by JBL to know and preserve the orchid diversity in Costa Rica was not constant in the beginning. In its early stages, JBL established collaboration with the Chicago Field Museum of Natural History, Harvard University Herbaria, Marie Selby Botanical Gardens, University of Florida, and Royal Botanic Gardens, Kew, among others (Atwood & Mora de Retana 1999; Pupulin & Romero 2003). These projects enriched the access to documentation for conducting orchid research in Costa Rica (Fig. 15). Early in this century, the JBL managed to consolidate its own research system, which laid the foundation for rapid development of its activities in Orchidaceae. This research system is based on some key elements: 1) botanical exploration and cultivation of specimens (Fig. 16); 2) comprehensive plant documentation including interpretation of the natural variation of populations, (Fig. 17); 3) a center with specialized equipment, trained staff, and students involved in the scientific projects; 4) a complex of well-documented collections (live plants, exsiccatae, spirit collections, tissues for DNA extraction, pollinaria, photographs and other digital documentation, botanical illustrations, etc.) (Fig. 18- 20); 5) an electronic system of interrelated databases; 6) complete access to literature and other original documentary



FIGURE 15. Type specimen of *Aspidia epidendroides* Lindl., collected in “Panama et Columbia occidentali” by H. Cuming in 1831. Reproduced with permission by the Board of Trustees, Royal Botanic Gardens, Kew.

sources concerning the taxonomy of Neotropical orchids; 7) a commitment to the policies of natural resource conservation and a strict relationship with the Ministry of Environment of Costa Rica; and 8) a network of international scientific cooperation with leading botanical research centers around the world. Through this system the JBL has assumed the role of carrying out the critical study of the orchid flora of Costa Rica. The JBL archival documents for the interpretation of Neotropical orchids are available over the Internet (see the site Epidendra, [www.epidendra.org](http://www.epidendra.org)).



FIGURE 16. Field trip at Fortuna, Chiriquí, Panamá. Photo by Eyvar Rodríguez.

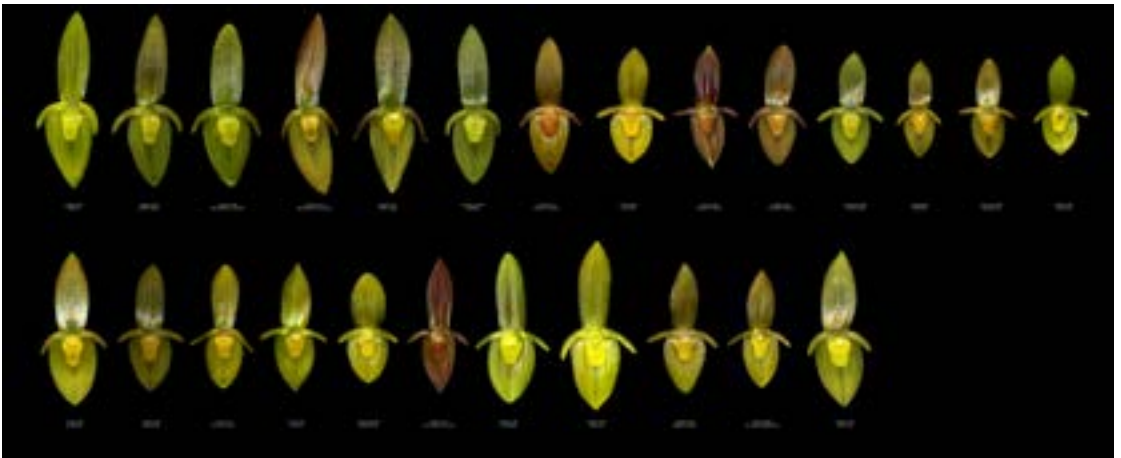


FIGURE 17. Comparison of the flowers of 25 specimens of *Pleurothallis homalantha* Schltr. showing the natural variation among individuals of different populations.

There are many advantages to developing orchid research in the tropics. The almost unlimited access to living material and wild orchid populations allow a more accurate assessment of intra- and interspecific natural variation (compared to the traditional practice of isolated expeditions and transfer of limited amounts of material to herbaria with a poor understanding of natural

variation). It is possible to obtain more comprehensive documentation by studying living organisms. Adequate documentation is a system that includes the preparation of materials such as herbarium specimens, spirit collections, photographs, color botanical plates, ink drawings, and images of pollinaria among others that, compared with protologues and type documents, allow



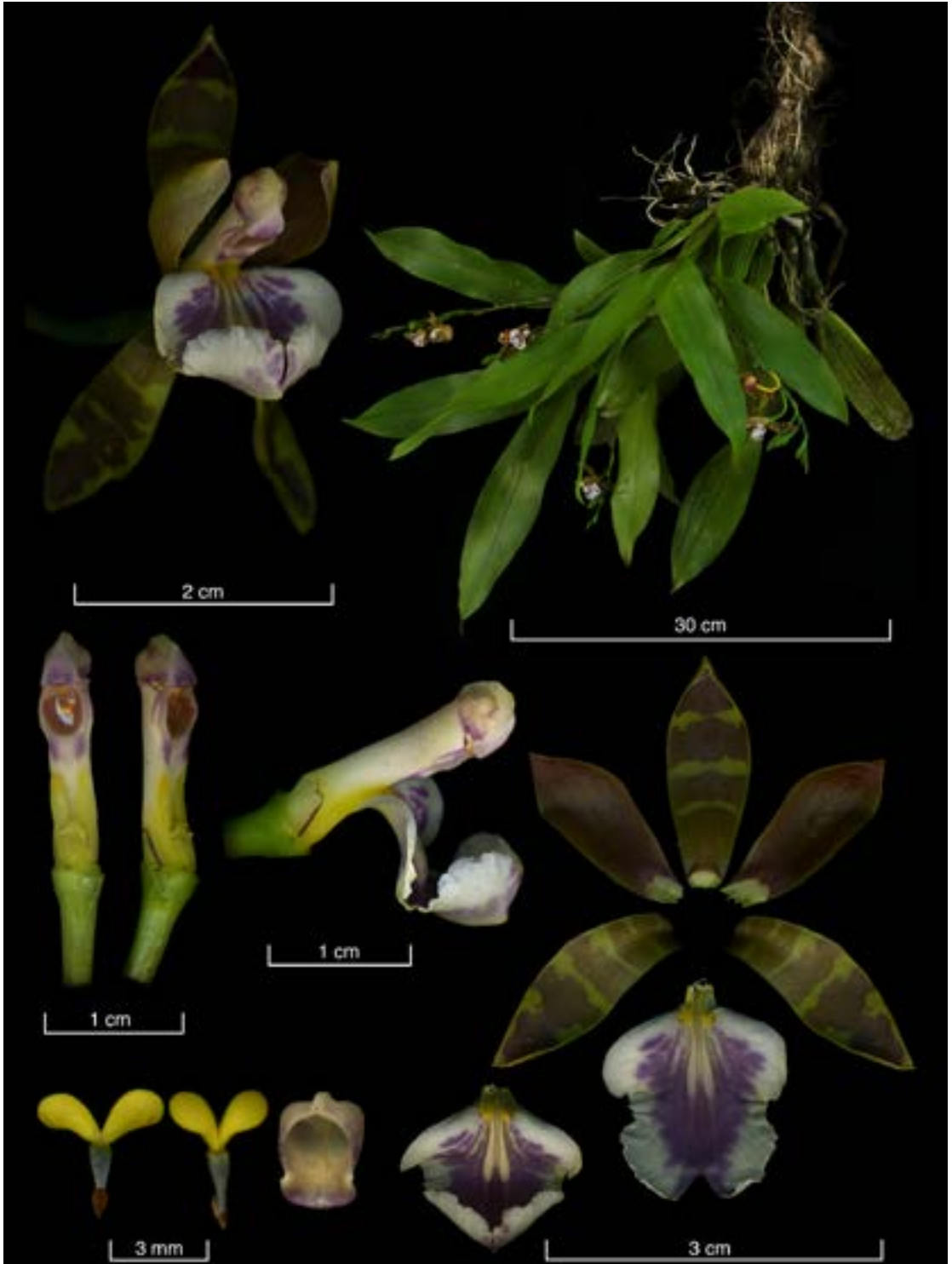


FIGURE 18. Botanical plate of *Aspasia epidendroides* from a plant collected in Chiriquí, Panamá. Courtesy of Herbario UCH.



FIGURE 19. Pollinarium of *Trichocentrum cebolleta* (Jacq.) Christenson, from the pollinaria collection of Herbario JBL.

the development of better taxonomic system (Fig. 15, 18). The development of electronic documentation techniques and the free flow of information through the Internet have contributed positively to increase scientific research (Pupulin 2007, 2009). Botanical exploration activities conducted in the past decade in Costa Rica, far from complete, yielded more than 100 orchid species new to science and several new records. This is a significant accomplishment for a traditionally well-studied orchid flora (Karremans *et al.* 2012).

#### The Orchid Project of JBL and UCH

The establishment of a permanent orchid research center in Panama is essential for the understanding of the flora of the southeastern Mesoamerican isthmus and its relationship with the Andean region. The Jardín Botánico Lankester of the Universidad de Costa Rica and the Herbarium UCH of the Universidad Autónoma de Chiriquí established a research center focused on the study of orchids. The main objectives are to perform a similar task in addition to that of JBL in Costa Rica and address the lack of an active center in Panama. With the adoption of an effective system of documentation of the diversity, JBL provided the methodology, information, and expertise for the long-term project on taxonomy

and systematics (Fig. 21). Essential documents and types that were repatriated in the last 10 years through agreements with other botanical institutions such as AMES, K, W, and MA are now available for the flora of Panama (Fig. 15). The first results including scientific papers and complementary information, photographs, specimens data, and literature of the project are available at EPIDENDRA.

There are many other reasons to implement this task in both countries. Their biological potential and geographical, geological, and floristic affinities are a challenge for science. Geographical proximity allows active communication between the two institutions. The continuous development of research activities, training of students, and reciprocal staff visits are some of the advantages. Their floras are complementary, and the understanding of the species distribution on both sides of the political boundary will lead to more precise conclusions. There are still large areas botanically unexplored. One of the most diverse and least known is the Cordillera de Talamanca, where the emblematic La Amistad International Park, a World Heritage site by UNESCO, protects almost 400,000 hectares of primary forest, the largest montane forest reserve in Central America (Fig. 22). In central and southeast Panama the



FIGURE 20. The spirit collection of Herbario JBL. The collection has almost 10,000 samples of Orchidaceae from the Neotropics.



FIGURE 21. Training of students from Universidad Autónoma de Chiriquí and Universidad de Costa Rica at Fortuna. Photo by Eyvar Rodríguez.

mountain ranges of Bagre, Sapó, Jurado, San Blas, and Darien are critical areas to explore. The proximity of the isthmus to South America also benefits the interpretation of the Andean orchid flora and its relationship to the northern Neotropics.

**ACKNOWLEDGMENTS.** We acknowledge the scientific services of Ministerio del Ambiente, Energía y Mares de Costa Rica (MINAE) and Sistema Nacional de Áreas de Conservación (SINAC) of Costa Rica and Autoridad Nacional del Ambiente (ANAM) of Panama for issuing the scientific permits of this project. This research was enabled by the Project 814-B2-161 “Hacia una moderna flora de orquídeas de Panamá” and by JBL and Herbario UCH. We thank Alec Pridgeon, José Portilla of Ecuagenera, Ltda., Ecuadorian Orchid Association, Guayaquil Botanical Gardens and Expoguayaquil who organized the 4th Scientific Conference on Andean Orchids in Guayaquil, Ecuador.

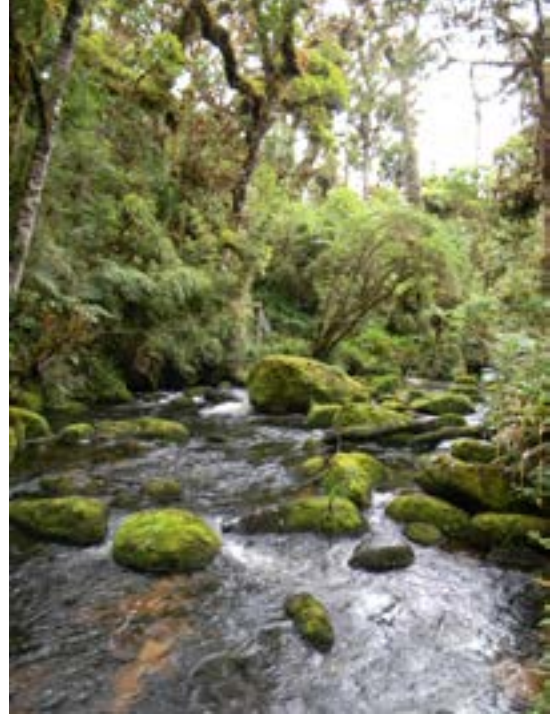


FIGURE 22. Río Terbi at La Amistad International Park. The river begins in Costa Rica and flows into Panama.

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## CONSERVATION STATUS AND STRATEGIES FOR THE PRESERVATION OF ORCHIDS IN THE SOCONUSCO REGION OF SOUTHEAST MEXICO

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**ABSTRACT.** A total of 320 orchid species are registered for the biodiverse region of Soconusco in the southeastern tip of Mexico. Given certain social, political, and legal limitations, the most productive strategy for preserving and restoring populations of the various species in danger of extinction, endemic, and over-exploited has been to work directly, intensively, and long-term with communities situated within or near to the few remaining hot spots of orchid diversity and abundance. With the appropriate permits, sustainable orchid cultivation has been established in various communities. We continue with long-term research into key aspects of the biology and ecology of orchids, identification of refuge sites, and modeling of geographical distribution of species.

**RESUMEN.** Un total de 320 de orquídeas se han registrado para la región del Soconusco en el extremo sureste de México. Dado los inherentes limitantes sociales, políticos y legales, parece ser que la estratégica más productiva para preservar y restaurar poblaciones de las varias especies en peligro de extinción, endémicas y sobreexplotadas es el trabajo directo, intensivo y a largo plazo con las comunidades ubicadas dentro o cerca de los pocos puntos calientes restantes de diversidad y abundancia de orquídeas. Con los debidos permisos, se han establecido cultivos sustentables de orquídeas en varias comunidades y se continúa con investigaciones a largo plazo sobre aspectos claves de la biología y ecología de las orquídeas, la identificación de sitios de refugio y la modelación de la distribución geográfica de especies.

**KEY WORDS:** modeling of geographic distribution, orchid mycorrhizae (OM), retrospective spatial analysis of pollination, Unit of Environmental Management (UMA)

Soconusco region is situated in the southeastern extreme of Mexico in the state of Chiapas, with the greatest orchid species richness per area in Mexico (Damon 2010). The region possesses highly fertile soils on the coastal plain and a diversity of ecosystems from mangrove swamps, through a variety types of tropical and temperate forest, to alpine dwarf forest on the slopes of the 4092 m-high Tacaná volcano. Of particular importance are the Tacaná – Boquerón biological corridor designated as a priority Terrestrial Region by CONABIO\* (RTP\*-I35) and the three Biosphere Reserves -- Tacaná Volcano, La Encrucijada and El Triunfo (CONANP\*).

Constant changes in agricultural priorities and the relatively recent paternalistic strategies, have promoted an increasingly demoralized, passive, and dependent population that now subsists on government aid channeled through a variety of social, economic, and environmental programs, while production, effective land management, and conservation of natural resources continue to deteriorate. Mexico is a wealthy country that could do much to satisfy the basic demands of the population and at the same time promote and support conservation, sustainable exploitation, and legal protection of its rich natural resources. Major problems are corruption and the lack

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\* GLOSSARY

CONABIO – National Commission for the Knowledge and Use of Biodiversity; CONANP – National Commission of Protected Areas; NOM-ECOL-059 – Legislation for the Protection of Mexican Native Species of Flora and Fauna; OSSSU – Orchid Seed Stores for Sustainable Use; RTP – Priority Terrestrial Region. CONABIO; SEMARNAT – Secretariat for the Environment and Natural Resources. Mexico; UMA – Environmental Management Unit.

of personnel, funding, training, and commitment to environmental issues.

Within that scenario, the trade in wild species is extensive, largely unregulated and an important and well-defended source of income for many rural peasants, which then feeds into the national and international networks. Unfortunately, although regulation and the identification of vulnerable species exists (NOM-ECOL-059-2010\*), with mechanisms for the issuing of permits for conservation management and sustainable exploitation of endangered species of flora and fauna, in reality most of the permits (UMAs\*, SEMARNAT\*) are authorized for hunting mammals and birds and there are few UMAs currently set up to commercially exploit plant and animal species that are not edible or huntable. Little has been achieved trying to work within the framework of the law to stop illegal extraction and trade in wild plants and animals or to halt habitat destruction.

The list of orchid species registered for Soconusco includes 320 species (Damon 2010), with several more awaiting identification and the resolution of taxonomic problems. At least 37 species are categorized as vulnerable or in danger of extinction within the NOM-ECOL-051-2010. Various species are endemic, and historic registers indicate that many species have now disappeared from the region. *Guarianthe skinneri* (Bateman) Dressler & W.E.Higgins, a once abundant but highly prized species, is now found only in private gardens and a few sites within protected areas and traditional coffee plantations (i.e. those with a diversity of original forest trees as shade).

Strategies for the preservation of orchids in Soconusco or anywhere else must, therefore, take into account social, political, and legal limitations. It appears that the best strategy is to work directly, intensively, and long-term with communities situated within or near the few remaining hot spots of orchid diversity and abundance. This labor-intensive, often frustrating approach depends on volunteers and a constant input of small amounts of money and materials, but it is to be preferred over short-term, government-financed projects that definitely do not work. It is also important to identify the few genuinely interested personnel within the relevant government departments and seek their collaboration for obtaining contacts and permits, in this case for the UMAs and the collection of orchids within protected areas.

The project "Ecology and Sustainable Cultivation of Soconusco Orchids" (ECOSUR-Tapachula) has set out to make a positive contribution to the preservation of native orchid species. We have identified pollination and mycorrhizal associations as two key areas of research, with training and long-term collaboration with rural communities (Damon 2006) for the sustainable exploitation of local orchids as the only practical activities that might make a difference. The project includes a botanical garden at 80 m and an orchidarium at 1200 m for the rescue, conservation, and study of local orchids (and other plants).

In collaboration with personnel from CONANP, two UMAs for the sustainable production of orchids in rural communities in Soconusco have been established with another in process and various other communities interested in participating. The participants start out with a week of introductory training before work begins, which also serves to filter out those who are only there in the hope of financial benefits or are not willing to participate actively. The methods applied in these UMAs involve the rescue of orchid plants found on the ground, on dead and felled trees or pruned branches (e.g. of shade trees in coffee plantations) and their establishment in specially designed galleries. With time and a regime of basic care, these plants are vegetatively reproduced and the divisions planted in suitable sites in traditional coffee plantations, forest fragments, and protected areas. A proportion of the flowers produced will be naturally or hand-pollinated to produce seed for local research and *in vitro* production in our project laboratory and to contribute to the OSSSU\* initiative (Seaton 2011); other flowers will be harvested and processed to make original craft items for sale for the benefit of the producers.

To be able to restore and preserve natural resources we need high-quality information about all aspects of biology, ecology, and refuge sites such as mountain peaks and ravines. In the case of orchids, especially epiphytic species, pollination and mycorrhizal associations are particularly important as they represent the two major bottlenecks in the orchid life cycle (Ackerman & Zimmerman 1994). We know little about pollination under natural conditions of a majority of the world's epiphytic orchid species, which are often inaccessible in the crowns of tall, tropical forest trees. Many species are rare, with small, widely dispersed

populations. Direct observation of the flowers of most species yields little information, as pollinator visits are unpredictable, infrequent, and ephemeral. The study of orchid pollination has been dominated by few studies of charismatic, widespread, accessible, and often terrestrial species that may not be representative of the majority. Furthermore, the orchid family is notorious for a diversity of pollination mechanisms, particularly various types of deceit strategies with particularly low pollination rates.

Low-percentage pollination of flowers may be normal, or even strategic, for many epiphytic orchids, and we may advance our understanding further if we rationalize these observations at a metapopulation level. I have devised a technique for studying the pollination of epiphytic orchids without direct observation of pollinators (Damon & Valle Mora 2008; Pérez-Hernández *et al.* 2011). This technique is adaptable to any orchid population where all flowering individuals may be observed. First, the precise conditions and spatial distribution of every flower are noted and mapped, followed by mapping fruits as they appear within that arrangement. The conditions noted may include visibility, orientation, height above ground, and the density of flowers at that point to analyze the importance of these parameters for the attraction of pollinators and the successful pollination of individual flowers or aggregations of flowers. The results may indicate pollinator preference for certain conditions, or in the case of deceit mechanisms, the likelihood of achieving pollinator deception within the range of flower presentations offered. However, results obtained with two miniature orchid epiphytes indicate that none of the parameters so far studied has a significant effect (density or aggregation of flowers occasionally has a weak effect) upon the likelihood of a flower being pollinated. I have suggested that, at least for the two species we studied, the pollination of any particular flower may depend on luck or chance, but that every successful pollination event is heavily compensated by the production of thousands or millions of seeds. We hope to continue with basic pollination studies (e.g. Damon *et al.* 2012) and in particular to develop this retrospective spatial technique, using improved geopositioning, mapping, and modeling techniques to extend it to a metapopulation level. However, recent directives that coffee producers

must eliminate all mosses and other epiphytes from the stems and branches of their coffee bushes have resulted in the almost total elimination of the small and miniature orchids that have so successfully adapted to coffee plantations since the end of the nineteenth century when coffee was first introduced into Mexico. As a result, it may now be impossible to continue with this interesting and revealing line of research, which would be impossible to carry out effectively within a forest environment.

The evolution and geographical distribution of Orchidaceae depend not only upon pollinators but also upon their endophytic, mycotrophic symbionts (Waterman & Bidartondo 2008), apparently involving a specific group of fungi, termed orchid mycorrhizae (OM) which belong to the *Rhizoctonia* group (Otero *et al.* 2007). Although it was originally thought that colonization was sporadic and seasonal (Hadley & Williamson 1972; Lesica & Antibus 1990), recent studies indicate that a dense and continuous colonization is the norm in tropical orchid species (Rivas *et al.* 1998; Pereira *et al.* 2005a). We now have evidence of patterns of evolutionary co-diversification and specificity (Otero *et al.* 2011), which could indicate that OM have a role to play in the rarity of many orchid species. However, to the contrary, a study of two European terrestrial species showed that questions of specificity did not determine rarity in those species (Cachapa Bailarote *et al.* 2012).

We know little about these endophytic mycorrhizal fungi that are implicated in various functions of the orchid plant throughout its life cycle, including seed germination, defense, and nutrition. Orchids can be classified within a continuous spectrum from partial (mixotrophy) to obligate mycotrophy, in which plants depend completely on the fungi for their supply of carbon and nitrogen (Julou *et al.* 2005). Orchid mycorrhizae have a positive effect on germination (Suárez *et al.* 2006; Rains *et al.* 2003), although Arditti (1992), Smith and Read (1997), and Zettler *et al.* (2003) have suggested reasons to doubt the importance of OM in this process. Studies on the symbiotic, *in vitro* germination of orchid seeds indicate that, although the seeds may imbibe water and swell in a pre-germinative phase, OM intervene in the process of differentiation and development (Zettler *et al.* 1998); without them the seeds remain trapped in the pre-germinative phase

and eventually die (Pereira *et al.* 2005b). Future work will focus upon the development of *in situ* symbiotic seed germination techniques, study of the ecological niches of these endophytic fungi, and research into the biochemical pathways that determine the process of recognition between fungus and orchid, invasion of orchid cells by the fungus, and control of the process of the digestion of fungal tissues by the orchid, which effectively functions as a parasite.

We are currently involved in a project to map the geographic distribution of all the species that have been registered within the Soconusco region, which in the initial stages involved collaboration with various institutions in Central America and Colombia (list of participating institutions provided on request; Damon 2012-13). This process has been useful to emphasize which orchid species are rare not only in Soconusco but also throughout their distribution, and also where investment and collaboration are needed to enable completion of vital taxonomic and ecological studies (Costello *et al.* 2013). The actual and predictive maps of geographical distribution generated will allow the identification of sites suitable for the restoration and conservation of populations, coincidence with pollinators and fungal symbionts, vulnerability to climate change, and much more.

Over the next few years we hope to be able to begin to answer some of the fundamental questions mentioned here, using remaining, common orchid populations, traditional coffee plantations, forest fragments, and protected areas as models (Light & MacConaill 2011). We hope it is not too late to save the majority of the Orchidaceae and the ecosystems that we and they depend on for future generations (Hirtz 2011).

**ACKNOWLEDGMENTS.** Thank you to Vincenzo Bertolini for contributing information about mycorrhizal interactions and to collaborators Nelson Pérez Miguel, James Rodríguez Acosta, the orchid producers, owners of the experimental sites, and various graduate and postgraduate students, for their hard work, ideas, and support.

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## ORCHIDS AND EMONOCOT – ASSEMBLING RESEARCH RESOURCES AND FACILITATING COLLABORATIVE TAXONOMY ONLINE

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**ABSTRACT.** The Orchidaceae is one of the eight families targeted for in-depth treatment in the groundbreaking eMonocot project. A consortium formed by RBG Kew, the Natural History Museum (London), and Oxford University, and funded by NERC, eMonocot is an e-taxonomy initiative that will provide the first web-based treatment for the world's monocot plants (constituting approximately 20% of all higher plants, some 70,000 species, and including numerous groups of the highest conservation, ecological, and economic importance). eMonocot will revolutionize the way taxonomic data are organized and accessed by both the practitioners and users of taxonomy, targeted at biodiversity and environmental scientists but also available to other users including volunteer biologists, horticulturists, schools, and the general public. Available information will include nomenclature, taxonomic descriptions, images, identification guides, as well as geographical, ecological, DNA sequence, and conservation data structured around a taxonomy derived from the World Checklist of Monocotyledons. As part of the eMonocot project, identification guides, and taxon pages to all Orchidaceae genera will be produced, alongside an interactive key and taxon pages for all Cyripedioideae (slipper orchid) species and infra-specific taxa. Researchers may upload content (such as images and species descriptions) to the taxon pages, edit the classification as taxa are described and revised, and access original publications for names and much more.

**KEY WORDS:** Orchidaceae, eTaxonomy, eMonocot, data sharing, web revision, collaboration

In an age of molecular data revolutionizing the classification of groups of plants such as Orchidaceae, taxonomy is as important as ever. Increasingly, plant taxonomists are working collaboratively with colleagues around the world and need to access and share resources such as checklists, original publications, herbarium records and specimens, bibliographic references, illustrations, photograph libraries, datasets, and distribution maps. Historically such resources have been held by institutions and ultimately accessible to the public or individuals working on the taxa only under carefully controlled conditions.

### **The distributed resources, online resources, and access for all**

Resources that document species' distribution, ecology, and evolution are scattered and often restricted to the world's major scientific libraries and specimen collections, most of which are located in Europe, the USA, and Australia. Consequently researchers

studying particular plant groups may need to travel extensively to access specimens and literature and collaborate with colleagues around the world. Several global initiatives have been addressing this issue by digitizing literature, specimens, and illustrations in collections and making them freely available on the Internet (e.g. Biodiversity Heritage Library, Global Plant Initiatives (GPI), Reflora Project). Other taxonomic resources, such as the International Plant Names Index (IPNI), Index Herbariorum (IH), and the World Checklist of Selected Plant Families (WCSP), are now available electronically and are continuously updated. While this shift to online platforms means that data are increasingly accessible, resources for particular taxonomic groups nevertheless remain diffuse.

### **The eMonocot project**

In order to facilitate the aggregation of these dispersed data, the eMonocot project is developing a freely accessible online information portal ([www.eMonocot.org](http://www.eMonocot.org)).

eMonocot.org) that provides a synthesis of high-quality scientific information about the world's monocot plants. Monocots were chosen for the focus of this project because they comprise some 70,000 species and represent 20% of all flowering plants, many of which are commercially and nutritionally important. Funded by the Natural Environment Research Council (NERC) via a consortium grant to the Royal Botanic Gardens, Kew, Oxford University, and the Natural History Museum (NHM), the eMonocot Portal is fast becoming a media-rich, interactive tool of discovery, education, and research for a broad audience from the biodiversity research disciplines to the interested public and is accessible free of charge to anyone with access to an Internet connection. Features include nomenclatural information, identification tools (including interactive keys), and informative taxon pages (including species descriptions, ecological data, images, and distribution maps), all brought together from reliable scientific resources.

#### **Entering data into eMonocot – classification, existing sources of data, and Scratchpads**

The initial 'backbone' classification for eMonocot is based on the WCSP, which provides taxonomic, nomenclatural, and geographic information for the ca. 90,000 accepted taxa included in the project (ca. 70,000 species plus intraspecific taxa). Additional data are brought together and displayed in the Portal from other source systems, which cover particular taxonomic groups in greater depth or provide different types of data. These include biodiversity data from Global Biodiversity Information Facility (GBIF) and e-taxonomy resources such as eFloras, CATE-Araceae, GrassBase, and Palmweb. Importantly, eMonocot is pioneering the development and use of new interactive, web-based e-taxonomy community research tools with Scratchpads websites ([www.scratchpads.eu](http://www.scratchpads.eu)) which enable researchers to manage, share and publish taxonomic data online while participating in virtual research communities in order to work collaboratively on the groups of organisms they study.

Scratchpads have been developed in partnership with the eMonocot project since 2010, having been funded since 2006 through the FP7 European

Union projects EDIT and ViBRANT. Scratchpad websites are modular content management systems, specifically designed to handle biodiversity content, including a wide range of file types (such as literature references, data sets such as DNA or morphological character matrices, and media, including photographs, video and audio, and specimen data). Scratchpads are free to set up and use, free for users to access, and are already being created and maintained by a wide variety of individuals and groups such as specialist taxonomic research groups, natural history societies, and biodiversity related projects such as the Sampled Red List Index (SRLI) and GPI.

Specifically eMonocot-themed Scratchpads have been created so that individuals and groups of individuals interested in contributing to eMonocot can create taxon-focused websites. These sites will bring together authoritative taxonomic expertise and resources and feed into the eMonocot Portal while also facilitating international collaboration and communication among scientists. The eMonocot Portal will display all of the data being fed into it from these Scratchpads, enabling all monocot data to be searched, analyzed, and downloaded from a single site (the Portal) using a range of additional tools and resources provided. A number of eMonocot Scratchpads have been established by the eMonocot project, supplemented by training workshops to enable monocot taxonomists to collate and share their research materials and data.

#### **Orchids and eMonocot**

Because of its sheer size and importance in terms of ecology and conservation, Orchidaceae is one of the eight families of monocots being targeted for in-depth treatment by the eMonocot project team. The Cyripedioideae Scratchpad (<http://cyripedioideae.e-monocot.org>) was among the first to be developed by the project (Fig. 1). Based on data extracted from published monographs of the subfamily written by Phil Cribb and enhanced with additional content gathered by members of the eMonocot project team, the site provides illustrated taxon pages for over 150 taxa in the subfamily (Fig. 2) and is complemented by an interactive key to all species, which was created using Lucid software (Fig. 3).





FIGURE 1. A screenshot of the Slipper Orchid Scratchpad homepage ([www.cypripedioideae.e-monocot.org](http://www.cypripedioideae.e-monocot.org)) displaying background information and media relating to the slipper orchids.

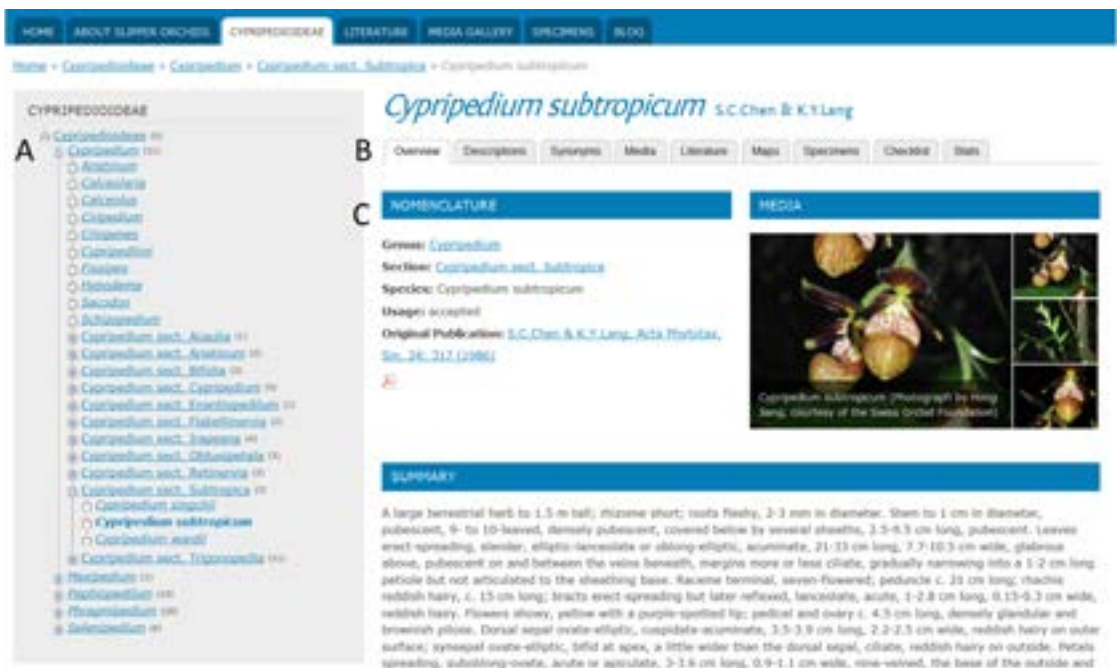


FIGURE 2. Example of a taxon page from the Slipper Orchid Scratchpad. A classification tree (A) allows the user to explore and navigate to a particular taxon. A series of tabs (B) on each taxon page allows the user to explore the different data pertaining to that taxon. For example, the overview page (C) provides nomenclatural information, including a link to the original description, media, and a summarized taxonomic description.

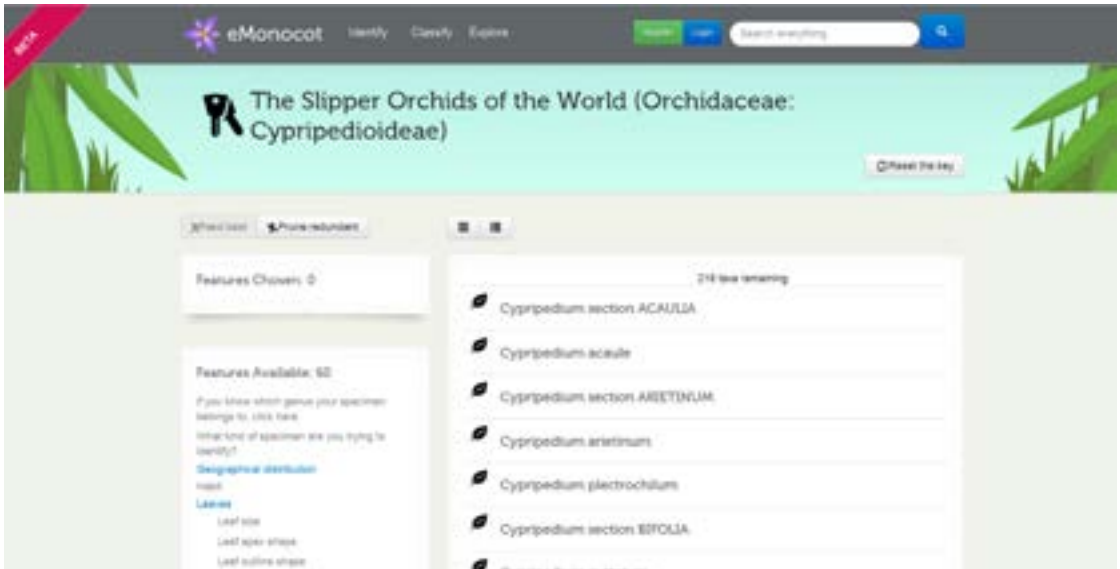


FIGURE 3. An interactive identification key to Cyripedioideae, created in Lucid and published in the eMonocot Portal.

With the support of Kew Publishing, Oxford University Press, and the editors of the series, eMonocot is also creating individual taxon pages for each orchid genus in the family based on descriptions published in *Genera Orchidacearum*. Taxon pages are being illustrated using images of digitized specimens, illustrations, and slides streamed directly from the World Orchid Iconography database of the Swiss Orchid Foundation – a collaborator of the project via a bespoke web service. Additional images can be added to any taxon page by registered users, allowing the display of personal and institutional photo galleries, subject to Creative Commons licensing (<http://creativecommons.org>) as chosen by the copyright owners.

#### Developing new orchid Scratchpads

A new research community Scratchpad site for Eulophiinae has been created by Ruth Bone (<http://eulophiinae.e-monocot.org>), who is actively seeking to engage researchers and field botanists working in Madagascar and southern and eastern Africa. The site is being used to lay a foundation for the taxonomic revision of the genus *Eulophia* and allied genera through compilation of protologues, eflora data (Flora Zambesiaca and Flora of Tropical East Africa), images, and specimen data. An associated research

project in collaboration with the Swiss Orchid Foundation is underway to develop a phylogenetic hypothesis for the group, and as work progresses the site will be used to document the research project via blogs and social media and make the project bibliography available. Scratchpads also integrate the new Pensoft writing tool that allows users to compile and publish taxonomic papers via the e-journals Phytokeys and the Biodiversity Data Journal; it is hoped that the Eulophiinae project will use these new tools. Another recently established eMonocot Orchidaceae Scratchpad is one for Aeridinae, which is being supplied with content (concentrating initially on descriptions, pdfs of original publications, and images) by Lauren Gardiner.

#### How eMonocot can help you – and how you can help be part of eMonocot

The eMonocot Project would like to encourage members of the orchid community to set up and maintain more Orchidaceae Scratchpads. Scratchpads can cover different levels of taxonomic groups depending on user interests and expertise and the needs of the communities involved at the level of subfamily (Cyripedioideae Scratchpad), tribe, subtribe (such as the Eulophiinae and Aeridinae Scratchpads) or genus. Scratchpad users

can manage their orchid data and resources, aiding their own research, while contributing data to the eMonocot project. If you would like to get involved with the eMonocot Project by joining or forming a scratchpad community, or wish to find out more about the project, please contact [enquiries@e-monocot.org](mailto:enquiries@e-monocot.org).

**ACKNOWLEDGMENTS.** We wish to show our sincere gratitude to the members of the eMonocot team from Kew, Oxford University, and the Natural History Museum for their hard work and dedication to the project. The Swiss Orchid Foundation is gratefully acknowledged for their provision of images to the Orchid scratchpad sites. Lauren Gardiner would like to thank Alec Pridgeon and the Organizing Committee of the 4th Scientific Conference on Andean Orchids for inviting her to speak at the meeting, and particularly to Pepe and Alex Portilla and their families for their hospitality after the conference.



## LA PESADILLA DE LINDLEY - LA BIOLOGÍA SEXUAL DE *CATASETUM* Y *CYCNOCHES*

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**ABSTRACT.** The sexual dimorphism of the genera *Catasetum* and *Cycnoches* is now common knowledge to orchid growers and investigators. Female flowers of *Catasetum* are all non-resupinate with a hooded lip, whereas the male flowers can be non-resupinate with a hooded lip or resupinate with a differently shaped lip, depending on the species. Only with male flowers present can the species of a *Catasetum* can be determined. In *Cycnoches*, flowers of both sexes are resupinate with a slender and long, curved column, resembling a swan's neck. The lip of male flowers of *C. sect. Heteranthae* is provided with finger-like projections, but female flowers of that section and those of *C. sect. Cycnoches* have an entire lip. Female flowers of both genera observed in the Botanical Garden of Munich bear pollinaria, but they are without any function. Slight movements of the female inflorescences of *Catasetum* cause the pollinaria to fall off, and as a result their presence is often overlooked. In female pollinaria of *Catasetum* the caudicles are absent, making pollination impossible in nature. Their pollen is germinable, and application of the pollinia to the stigma of the same flower yields viable seeds. The seldom-observed intermediate forms have a stigmatic cavity as in the male flowers. Their pollinaria are similar to those of the female flowers, so they are also non-functional. They do not serve either as males or females. In female *Cycnoches*, the anther cap does not fall off, so preventing pollination by such a pollinarium. The pollinia are encapsulated by the anther cap and so cannot come into contact with the stigma of another flower. Here, too, germination tests of the female pollen were positive. It is suspected that selfing of the female flowers here also will result in viable seeds. In both cases the female flowers are true hermaphrodites, but because of their limited male function one should think of them as functional female flowers. Male flowers, however, do not develop precursors of ovules, and a stigma is not present. Given the different flower morphologies and the pollination processes, one assumes that the floral fragrances should differ among the sexes of *Catasetum* flowers. The battered pollinator will remember the negative experience with the male flowers. So different fragrances in the female flowers would be the way out of this dilemma. Floral fragrances of five *Catasetum* species and two *Cycnoches* species were investigated by gas chromatography. Only weak differences in the fragrance composition of the separate sexes were detected, begging several questions. What are the forces driving the pollinator from male to female flowers? Is the weight of the pollinarium sufficient to explain avoidance of the male flowers? Does the bee still remember the disagreeable episode? Is identification effected only by visual senses?

**KEY WORDS:** Orchidaceae, sexual dimorphism, floral fragrances, pollination biology, *Catasetum*, *Cycnoches*

### Introducción

El dimorfismo floral de los géneros *Catasetum* y *Cycnoches* sect. *Heteranthae* es marcado y bien conocido en el mundo de los científicos y cultivadores. En *Cycnoches* sect. *Cycnoches* y en *Mormodes* es menos pronunciado, pero también conocido.

Las flores femeninas de *Catasetum* son muy similares en todas las especies: son no resupinadas y el labelo, siempre en forma de yelmo, está colocado arriba de la columna. Las inflorescencias en casi todas las

especies son erectas y su raquis es rígido. Las plantas de *Catasetum* que presentan sólo flores femeninas no se pueden determinar. Las flores masculinas, por otro lado, son bien diversas en su morfología; pueden ser resupinadas o no y tienen mayormente inflorescencias arqueadas. Raras veces su labelo tiene forma de casco, mientras que el resto puede tener labelos planos, y en muchos casos mostrar emergencias extrañas. La columna de las flores masculinas tiene siempre dos antenas (menos pronunciadas en el subgen.

*Pseudocatsetum*), carácter del cual deriva el nombre *Catsetum* (del Griego *kata*, “inferior”, y del Latin *seta*, “cerda o hebra”). Solamente teniendo flores masculinas a mano se puede determinar la especie!

Lo mismo se aplica a *Cynoches* sect. *Heteranthae*, en las que solamente con flores masculinas se pueden diferenciar las especies. Las flores femeninas de esta sección tienen una inflorescencia corta, erecta y rígida, el labelo es entero y se encuentra arriba de la columna. Las masculinas también son muy diferentes, con inflorescencias largas, delgadas y péndulas, mayormente provistas de proyecciones en forma de dedos. El labelo en este grupo está posicionado arriba de la columna (a diferencia de *Catsetum*!) pero como las inflorescencias son péndulas, las flores son resupinadas (igual que en *Catsetum*!). La morfología floral tan compleja, junto con el dimorfismo floral de ambos géneros, ha sido la madre de la confusión de su *status* desde los primeros días, y por esta razón se presenta aquí una breve sinopsis de su historia.

### Historia

La primera especie, *Catsetum macrocarpum*, fue descrita por Kunth en 1822 después de ser propuesta por L.C. Richard. Las flores de ambos sexos de esta especie tienen el labelo en forma de yelmo y no están resupinadas, así es que la diferencia entre ellas es leve. En el tiempo de John Lindley el género todavía era un misterio porque solo pocas plantas eran disponibles para estudio. En el año 1832 Lindley describió los dos géneros *Monachanthus* y *Myanthus*, constatando que eran muy afines por su hábito, “the fleshy stems are exactly those of *Catsetum*, as the leaves, except that they are shorter.” (Lindley 1832). Richard Schomburgk hizo observaciones de campo en Demerara [Guyana] notando que las inflorescencias de *Myanthus* y *Monachanthus* crecían en la misma planta (1829/30). Así mismo, tanto H. Crüger [Trinidad] como Fritz Müller (1868) [Brasil], notaron que *Catsetum* nunca presentó frutos, mientras que las plantas de *Monachanthus* cargaban cápsulas enormes (Fig. 1). Darwin, en su segunda edición de su obra *The Various Contrivances by which Orchids are Fertilised by Insects* (Darwin 1862) y “On the three remarkable sexual forms of *Catsetum*” (Darwin 1862), solucionó el enigma, mostrando que *Monachanthus* representaba la forma femenina de



FIGURA 1. *Catsetum viridiflavum* (Panamá). Planta grande y vigorosa, cargando una cápsula enorme y una inflorescencia masculina. Foto: G. Gerlach.

*Catsetum*. Rolfe (1891) finalmente terminó de resolver el problema, elucidando algunos errores hechos por Darwin.

El género *Cynoches* fue establecido por Lindley (1832), basado en una flor masculina de *C. loddigesii*. En 1836 él recibió flores algo diferentes de la primera, y la describió como *C. cucullata* (la cual, en realidad, sería la forma femenina de *C. loddigesii*). Más tarde, la investigación de más flores provenientes de un envío de Surinam, le indujo a concluir que tanto *Cynoches* como *Catsetum* eran parte de una “sportive condition”, que significa que son capaces de producir diferentes flores en diferentes periodos. En realidad, su *C. cucullata* representaba el sexo femenino de su *C. loddigesii*, la cual pertenece a la secc. *Cynoches* en la que las flores masculinas y femeninas son similares. La historia sigue y culmina en la otra sección, con muchas más curiosidades.



James A. Bateman recibió del comerciante inglés George Ure Skinner, establecido en Guatemala la noticia de la existencia de una *Cycnoches* con inflorescencias largas y péndulas (hasta entonces se conocía de América Central solamente *C. ventricosum*, una especie de la secc. *Cycnoches* con inflorescencias cortas). Bateman pidió algunos especímenes, pero cuando estos empezaron a florecer, la primera planta fue identificada como *C. ventricosum* por su inflorescencia corta. Asumiendo que los especímenes que había recibido de Guatemala hubieran sido confundidos, Bateman pidió otra vez plantas de la especie con inflorescencias largas. Con éstas pasó lo mismo que con las anteriores. En una tercera petición Skinner, quien iba de regreso a Inglaterra, supervisó personalmente el transporte. Durante el viaje una planta floreció con inflorescencias largas, así que Skinner estuvo seguro de evitar una nueva confusión. Finalmente Bateman recibió la planta tanto buscada, la cual nombró *C. egertonianum* (1842), pero cuando floreció nuevamente, quedó consternado frente a unas flores totalmente diferentes a las que acababa de describir como *C. ventricosum*. Como la primera inflorescencia estaba todavía presente en la planta, Bateman no podía explicarse este fenómeno de dos tipos de flores al mismo tiempo. En esa época ninguna persona era capaz de elucidar esta situación, y fue nuevamente Darwin (1877) quien resolvió el problema (véase también la historia más detallada en Carr 2006).

Las plantas de *Catasetum* y *Cycnoches* producen más inflorescencias con flores masculinas que femeninas, ya que las plantas más jóvenes y pequeñas no tienen suficientes reservas para generar las enormes cápsulas propias de estos géneros. Otro factor que determina el sexo de las flores es la luz que recibe la planta. Las plantas que crecen en sitios bien sombreados no producen flores femeninas. La producción de flores femeninas es dependiente de los recursos del ambiente: las reservas acumuladas por el tamaño de los bulbos, la luz, y el acceso a nutrientes (Gregg 1977, 1978, 1982). En la naturaleza uno puede observar plantas grandes que cargan cápsulas ya que éstas necesitan una gran cantidad de energía para madurar – así, las condiciones ambientales controlan la expresión sexual. Las buenas condiciones en los viveros del Jardín Botánico de Munich permitieron que las plantas de *Catasetum* y *Cycnoches* produjeran flores femeninas, requisitos



FIGURA 2. *Catasetum schmidtianum* (Brasil). Flor femenina con la antera todavía presente. Se nota el viscidio marrón, no pegajoso. Foto: G. Gerlach.

ideales para investigar de nuevo los resultados de las investigaciones anteriores. No solo las flores machos y hembras pudieron ser observadas, sino también formas intermedias entre ellas. Todas las flores femeninas de ambos géneros tenían polinarios (Fig. 2), pero sin función reproductiva. Movimientos leves de las inflorescencias causan la caída de los polinarios de las flores, y en el ambiente natural de las plantas, por la presencia del viento, los polinarios nunca fueron encontrados. La facilidad de pérdida de estos polinios no fue notada en trabajos pasados.

### *Catasetum*

La morfología de la columna de las flores femeninas y masculinas es bien diferente (Fig. 3, 6). La flor femenina siempre tiene el ovario bien desarrollado, su labelo es no resupinado y tiene forma de yelmo, su columna es bien compacta y corta con el estigma



FIGURA 3. *Catasetum fuchsii* (de Bolivia). Caso raro de una inflorescencia con flor femenina (abajo) y flor masculina. Se ve que la flor femenina tiene el labelo en forma de casco y no es resupinada (labelo arriba de la columna), mientras que la flor masculina (arriba) es resupinada y tiene el labelo bien diferente. Foto: G. Gerlach.

ventral en forma de hendidura transversal y lleno de un líquido o una jalea bastante viscosa. La antera es apical, el polinario presenta un viscidio redondo marrón, un estípote corto y dos polinios que no se salen de la capucha de la antera (Fig. 2). Los polinios siempre son más pequeños en las flores femeninas que en las masculinas (Fig. 7).

Las flores masculinas no tienen el ovario desarrollado, son resupinados o no. Su labelo, en pocos casos, tiene forma de casco, su columna es delgada y larga con la cavidad estigmática bien abierta y seca. Esta cavidad está en parte cubierta por dos antenas sensibles al contacto que sobresalen. La antera consiste en una capucha bien desarrollada, en muchos casos con una emergencia apical en forma de punta larga que cubre el polinario. El polinario se divide en un viscidio bien



FIGURA 4. *Catasetum juruenense* (Brasil). La inflorescencia carga dos flores hermafroditas (abajo) y una flor masculina. Nota que una de la hermafroditas es solamente resupinado por la mitad (app. 90°). Foto: G. Gerlach.

pegajoso de gomacolor blanco a crema, un estípote largo ligulado y dos polinios amarillos, ovales y parcialmente hendidos (Fig. 7).

El polinario de las flores tanto femeninas como masculinas tienen un viscidio, un estípote y dos polinios. En las femeninas faltan las caudículas que conectan los polinios con el estípote. Así, los polinios no sirven para el transporte del polen porque los polinios no se quedan pegados al polinizador. Experimentos sobre la viabilidad de los polinios muestran que el polen germina y los tubos polínicos se desarrollan. Si uno pone los polinios en una solución al 10% de azúcar, el polen germina al cabo de pocos días. La aplicación de polinios al estigma de la misma flor resultaba en la formación de cápsulas, y las semillas obtenidas de éstas germinan y finalmente se desarrollan hasta ser plantas (véase también Light 1995).





FIGURA 5. *Catasetum barbatum* (de Venezuela). En esta inflorescencia la flor masculina está abajo (derecha) y la hermafrodita arriba (izquierda). En las hermafroditas se notan antenas rudimentarias sin función. Foto: G. Gerlach.

Todas las formas intermedias (Fig. 4) observadas entre los sexos tenían la cavidad estigmática similar a la de las flores masculinas lo que significa que por estar seco los polinios no se adhieren. En algunos casos las flores tenían antenas rudimentarias sin función (Fig. 5), éstas antenas eran desde muy pequeñas y cortas hasta más largas, pero nunca llegaron al tamaño de las que poseen las flores masculinas. Los polinarios eran similares a los de las flores femeninas, ya que le faltaban las caudículas, las cuales en los polinarios masculinos conectan los polinios con el estípite. Así, quedaban también sin uso porque los polinios no pudieron fijarse sobre un probable polinizador. Estas formas hermafroditas no funcionaron ni como machos ni como hembras, eran totalmente inútiles para la reproducción sexual.

### *Cycnoches*

Como se mencionó anteriormente, hay dos secciones en *Cycnoches* que se diferencian profundamente en cuanto a sus flores masculinas.

Las femeninas tienen inflorescencias cortas con hasta 4 flores con el ovario bien desarrollado. Consisten en flores no resupinadas con el labelo entero, una columna intermedia más o menos compacta, en la cual el estigma es ventral en forma de hendidura transversal. Arriba del estigma, el clinandrio forma una cuchara que sirve para separar los polinios del polinario en el acto de polinización (Fig. 8). Finalmente en su punta carga una antera semiglobosa, y debajo de ella un polinario con el viscidio pegajoso, un estípite ligulado y dos polinios amarillos. Los polinios quedan siempre encapsulados por la capucha de la antera y por esta razón nunca llegan a tener contacto con el angosto estigma.

Las flores masculinas se encuentran en inflorescencias largas y delgadas, son resupinadas o no, y su ovario no está revelado. Su labelo puede ser entero y sésil o unguiculado provisto con proyecciones en forma de dedos. La columna es larga y muy delgada, terete, curvada, dilatada y subclavada en el ápice. Ésta termina en un clinandrio con dos proyecciones



FIGURA 6. *Catasetum tenebrosum* (de Perú). Inflorescencia con flores femeninas (abajo) y masculinas (arriba). Foto: G. Gerlach.

rostradas, debajo de los cuales se encuentra el polinario con su capucha de la antera. El polinario consiste en un viscidio bien pegajoso, grande y elíptico, un estípito ligulado y dos polinios amarillos esféricos.

En los polinarios de flores femeninas la gorra no se cae, impidiendo la polinización por medio de un tal polinio. Experimentos de germinación con polinios de flores femeninas en solución de azúcar dan resultados positivos, lo que significa que el polen es viable. No se realizaron experimentos de polinización con polinarios de flores femeninas, pero se supone que darían resultados iguales al del género *Catasetum*.

En *Cynoches* se observaron menos flores hermafroditas, pero éstas observaciones no se pueden comprobar por estadística. Así como en *Catasetum*, resultaron inútiles para la reproducción sexual. El labelo en estas formas siempre es entero, pero en su lámina tiene procesos dactilares reducidos (Lámina 1).



FIGURA 7. *Catasetum sanguineum* (Venezuela). Polinarios de flores masculinas grandes (izquierda) y femeninas pequeñas (derecho). La antera de la flor masculina en forma de gorra es alargada (izquierda abajo) la de la femenina subhemisférico (centro). Como las caudículas no están desarrolladas, los polinios (amarillo en el centro) no están conectados con el resto del polinario consistiendo en viscidio y estípes (dos partes marrones a la derecha). Foto: G. Gerlach.



FIGURA 8. *Cynoches maculatum* (Venezuela). Columna compacta de la flor femenina. El polinario a dentro de la antera está todavía presente. Los polinios están totalmente cubiertos por la antera, el estípes ligulado es visible y se nota también parte del viscidio con la pega. Nota las dos cucharas del clinandrio a la izquierda y derecha de la punta de la columna. Foto: G. Gerlach.



LÁMINA 1. *Cycnoches maculatum* (Venezuela). Tres formas de flores. Flor masculina (izquierda), flor hermafrodita (centro) y flor femenina (derecha). La flor hermafrodita muestra un estatus intermedio en su columna (más compacta que en la masculina) y en el labelo (procesos presentes, pero mucho más cortos que en la masculina). Fotos: G. Gerlach.

En los dos casos de *Catasetum* y *Cycnoches* las flores femeninas son bisexuales, pero su función masculina es limitada; es más apropiado definir las como flores funcionales femeninas. Las flores masculinas, por otro lado, no tienen los precursores de los ovulos, no tienen el estigma mojado y con ninguna artimaña se pueden provocar la producción de semillas: ellos son exclusivamente machos.

### Biología reproductiva

*Catasetum* y *Cycnoches* tienen flores que muestran el síndrome floral de perfumes, lo que significa que son polinizados por abejas euglósidas machos, quienes buscan y colectan los perfumes florales. Estos machos no colectan cualquier sustancia aromática, sino que buscan ciertos componentes muy especiales en el aroma de estas flores. Cada especie de abeja tiene un *bouquet* específico. Cuando el polinizador de una especie de *Catasetum* visita una flor masculina y, al colectar, toca la antena sensible, la flor catapultará el polinario que queda adherido sobre la espalda del polinizador (en *C.* subgenus *Pseudocatasetum*, es colocado sobre el pecho). El polinario vuela por el aire, elegante como un atleta de barra fija, porque tiene todavía la capucha de la antera como estabilizador de vuelo. El choque con el cuerpo de la abeja es muy fuerte porque el polinario es grande y por esta razón pesado. Los polinarios de *Catasetum* se hallan frecuentemente sobre abejas euglósidas, pero es sumamente raro que se encuentren más que uno. Por otro lado, las abejas con polinarios de *C.* subgenus *Pseudocatasetum* tienen de vez en

cuando más que un polinario sobre su pecho.

Si uno tiene a la vista las diferencias morfológicas entre las flores femeninas y masculinas de *Catasetum* y *Cycnoches*, se impone la idea de que los aromas florales deben también ser distintos entre los sexos. El polinizador, que ha sido golpeado por el polinario de la flor masculina de *Catasetum*, supuestamente recuerda su mala experiencia con estas flores por un cierto tiempo (Romero & Nelson 1986). Él no quiere repetir su experiencia “traumática”, pero necesita las sustancias del aroma floral urgentemente para su boda. ¿No sería un escape a este dilema si las flores femeninas produjeran un aroma floral diferente? Los aromas florales de nueve especies de *Catasetum* (*C. bicolor*, *C. cernuum*, *C. fuchsii*, *C. juruenense*, *C. pileatum*, *C. planiceps*, *C. sanguineum*, *C. schmidtianum* y *C. tenebrosum*) y dos de *Cycnoches* (*C. pentadactylon* y *C. powellii*) fueron investigados por cromatografía de gases, pero se detectaron solamente leves diferencias en la composición (para evitar problemas con la variabilidad dentro de una especie, para la investigación se tomó una sola planta que produjo flores de los dos sexos en diferentes momentos). Todavía no sabemos mucho sobre la detección de los aromas florales por parte de los euglósidos. Experimentos de campo en su hábitat con la sustancia preponderante de *Coryanthes albertinae*, el undecatrieno, resultaron negativos, lo que significa que esta sustancia no es un atrayente para el macho euglósido. Al mismo tiempo, estas abejas llegaron a cebos de otras sustancias, entre las cuales habían 13 ejemplares con polinarios de *C. albertinae*. En el

momento, no pudimos explicar este comportamiento, pero años después detectamos una sustancia presente en concentraciones mínimas: el (Z)-2,6-dodecadien-1,5-olido, el cual era un atrayente muy fuerte. ¿Podría ser posible que en *Catasetum* y *Cycnoches* ocurra una situación similar? En *Cycnoches* también existe un mecanismo expulsivo de los polinarios en las flores masculinas. Normalmente el polinizador empieza a coleccionar el perfume en la lámina del labelo. El macho euglosido se agarra en los procesos dactilares, pero con su peso el labelo se mueve hacia la columna la cual se encuentra por debajo del mismo. Al tocar la punta de la columna el polinario se dobla rápidamente y el viscidio se pega sobre el abdomen del insecto. El proceso es muy rápido y pasa con mucho fuerza, produciendo un estado de shock en la abeja. El polinizador se asusta y se va de la flor. La revisión de varias colecciones entomológicas nunca reveló abejas con mas que un polinario, y eso significa que el trauma después de la visita de una flor masculina es fuerte y que éste individuo nunca mas visitó una flor masculina. Este

efecto es un mecanismo muy efectivo para evitar la autopolinización, ya que obliga a la xenogamia, la cual es el mecanismo preferido en las flores.

Aquí se concretan las preguntas: ¿Cuál es la motivación del polinizador para volar de las flores masculinas a las femeninas y efectuar la polinización? ¿Es el peso del polinario que carga la abeja por si solo suficiente para evitar la visita a otras flores masculinas? ¿Por cuánto tiempo la abeja recuerda su episodio desagradable? ó ¿Son los recuerdos visuales los que hacen que eviten otra flor masculina?

AGRADECIMIENTOS. Primeramente a mi esposa por corregir y discutir el texto presente, a Gustavo Romero por sus valiosas discusiones al respecto de las *Catasetinae*, Bert Klein y sus jardineros por cultivar las plantas tan bien que florecieron en muchas ocasiones femeninas, a Roman Kaiser por analizar las aromas florales de las diferentes especies de *Catasetum* y *Cycnoches*, a Jutta Babczinsky (Asistente técnica) por coleccionar las aromas de las flores y otra asistencia técnica y todas las numerosas personas en América Latina que me ayudaron con cariño y calor en mis salidas de campo.

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## LONG-TERM HURRICANE EFFECTS ON POPULATIONS OF TWO EPIPHYTIC ORCHID SPECIES FROM GUANAHACABIBES PENINSULA, CUBA

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**ABSTRACT.** Disturbances affect the spatial and temporal dynamics of ecosystems. The lack of long-term demographic data affects our understanding the effects of high intensity disturbances such as hurricanes. In this paper we assess population recovery of two epiphytic orchids, *Broughtonia cubensis* (Lindl.) Cogn. and *Dendrophylax lindenii* (Lindl.) Bentham ex Rolfe, for 7-8 years after Hurricane Ivan (September 2004, category 5, Saffir-Simpson scale). To analyze some spatial components, we applied recent techniques of marked point pattern analysis to ask two questions: 1) at level of phorophytes, was the height and basal diameter (DBH) related to the damage caused by hurricane Ivan, and 2) was the height of epiphytic orchids on phorophytes related to the damage, too? We found that phorophyte damage caused by the hurricane depends on the ecosystem in which the trees were found. Furthermore, damage to individuals of *B. cubensis* was independent of their height above ground, in contrast to those of *D. lindenii*. To assess orchid recovery we compared pre- and post-hurricane measures of vegetative traits: number of leaves and leaf area for *B. cubensis* and number of living roots and mean root length for the leafless *D. lindenii*. We also compared changes in the production of inflorescences, flowers, and fruits over this recovery period. We found that vegetative parts of *B. cubensis* recovered, whereas those of *D. lindenii* did not. However, neither reproductive effort nor success returned to pre-hurricane levels for either species. The greatest decline in fruit set was by *D. lindenii*, which may reflect a significant decline in availability of their sphingid pollinators. The relative population growth rate from 2004 to 2012 for *B. cubensis* indicates that the population has stabilized. Unfortunately, analysis of our eight-year data set (2003-2011) for *D. lindenii* shows negative population growth. Despite an evolutionary history exposed to severe periodic storms not all orchid species are resilient. .

**KEY WORDS:** epiphytes, hurricane recovery, Orchidaceae, Caribbean

Orchidaceae is one of the largest flowering plant families and is primarily threatened by human activity and habitat changes (Kindlman *et al.* 2002). With global warming, the frequency and severity of storms including Caribbean hurricanes is expected to rise, which can cause widespread habitat destruction.

The role of disturbance in affecting the spatial and temporal dynamics of ecosystems is increasingly recognized as a dominant theme in ecology. Understanding the effects of infrequent and high-intensity disturbances such as hurricanes is difficult, especially in tropical environments where there is a lack of long-term demographic data (Willig *et al.* 2011). The Caribbean Basin is characterized by high

cyclonic activity (Walker *et al.* 1991; Landsea *et al.* 1999), with over 2000 hurricanes recorded from 1851 to 2008. Consequently, the Caribbean Basin is an environment in which disturbance regimes likely produce a sequence of events in which cumulative or interactive effects play a dominant role in determining the abundance and distribution of organisms (Waide and Lugo 1992; Willig *et al.* 2011).

Caribbean hurricanes have affected ecosystems (Lodge & McDowell 1991), plant population dynamics (Lugo *et al.* 1983), food webs (Spiller and Schoener 2007), and nutrient cycling (Sanford *et al.* 1991). Especially threatening are the effects of hurricanes on epiphytic orchid communities due to

phorophyte damage, which could result in the loss of large components of the community (Rodríguez-Robles *et al.* 1990; You & Petty 1991; Migenis and Ackerman 1993; Tremblay 2008). This exacerbates an already sensitive situation in which many species are threatened and endangered (Salazar 1996; Morales 2000) primarily because of collectors and anthropogenic habitat changes (Kindlman *et al.* 2002). Most studies indicate that future changes in hurricane frequencies in response to global warming will be region-dependent (Pielke *et al.* 2005). Thus, it is all the more important to understand the long-term effects of successive disturbances on the resilience of biological systems (Schoener & Spiller 2006).

Our general goal was to determine recovery patterns in tropical epiphytic orchids after a severe weather event. Specifically:

- Is the height and DBH of phorophytes relevant to the severity of tree damage?
- Is the positional height of orchids on phorophytes related to the degree of damage caused by Hurricane Ivan?
- Do species recover over a period of 7-8 years?
- Are patterns of recovery similar for orchid species subjected to the same disturbance?

### Materials and methods

*Study area* — The Biosphere Reserve “Península de Guanahacabibes” is located in the westernmost region of Cuba in the municipality of Sandino, Pinar del Río province. The peninsula is long and narrow, with flat areas covering approximately 101,500 hectares (Herrera *et al.* 1987) of which 28,000 belong to the Guanahacabibes National Park. Our research took place at Cabo San Antonio.

*The species* — *Broughtonia cubensis* is endemic to Cuba and has a high ornamental value. It is epiphytic, occurring in xerophytic vegetation of Guanahacabibes and in low mountain ranges of La Coca, Mayabeque province. It blooms between January and March, with a peak in February (Mújica *et al.* 2000). *Dendrophylax lindenii* is a large-flowered, ornamental epiphytic species native to Cuba and Florida (U.S.A.). Its leaves are bract-like and ephemeral, giving it a leafless appearance. Virtually all photosynthesis is by its extensive root system, presumably making it

vulnerable to habitat changes. In Guanahacabibes, *D. lindenii* inhabits semideciduous forests and elevations near sea level. It blooms mainly from October to December (Mújica *et al.* 2000).

*Data collection and analysis* — The xeromorphic coastal vegetation occupied by *B. cubensis* was intensively studied from March 2004 to March 2012 using 10 arbitrarily located transects (5-7 × 100 m). Where orchids were most common, we established five 20 × 20 m plots. For *D. lindenii*, we established two 10-15 × 1000 m transects in the semideciduous forest. On the sites with high occurrence of orchids we created three 20 × 20 m plots. All occupied trees were identified and mapped, and all orchid plants were individually marked.

For analysis of damage on phorophytes and number of individuals in each population, we used the information in the mapped plots. For analysis of vegetative and reproductive variables and relative population growth rates (RGR), we used transect data.

To determine the effect of Hurricane Ivan on orchid phorophytes, we assessed effects at two different levels: damage to phorophytes (orchid host-trees) and damage to the orchids. For phorophyte species, we established two patterns of damage: severely affected phorophytes (tip-ups or snap-offs) and phorophytes less affected (defoliation and branch breakage). We tested whether tree height or DBH influenced whether or not phorophytes were severely damaged.

Our orchid data included the number of individuals on each phorophyte and their height on the tree above ground. For *B. cubensis* we collected information on number of leaves and leaf area, and for *D. lindenii* we calculated the number of living roots and mean length of all living roots. Reproductive data for both species included the percentage of plants that failed to produce flowers, number and length of inflorescences, number of flowers produced per inflorescence, and the number of flowers and fruits. We compared traits before Hurricane Ivan, immediately afterwards (2004), and then eight years later.

Finally, we evaluated the resilience of the two orchid species after the hurricane by changes in the relative growth rate (RGR) and using transition matrix analyses after the impact on their populations (from 2006 to 2011 for *D. lindenii* and 2006-2012 for *B. cubensis*). Matrix analyses are the most powerful methods for predictive

analysis of population dynamics. For plants, stage-based matrix projection models (Lefkovich 1965) are more appropriate for analyzing plant population dynamics than age-based models (Caswell 2001 and references therein) because life-history stage (for example, seed, seedling, juvenile, adult, senescent) and size are more important than age in determining demographic parameters (Harper 1977). For the matrix analysis we structured the populations in four life stages on the basis of leaf area for *B. cubensis* and mean root length in *D. lindenii*. In plants it is common to use size as a variable to define life stages in a population. Other studies carried out in Central America use length of the most recently produced pseudobulb (Zotz & Schmidt 2006). However, in our study we decided to use leaf area of each individual to define the life stages in the population of *B. cubensis* following Mújica (2007). Annually we measured length and width of leaves of each individual to obtain their leaf area. In the case of the leafless *D. lindenii*, the only alternative was to calculate the mean length of all living roots on the individual plant.

*Statistics* — For the comparative analyses between the possible relationship of the height and DBH with the damage caused to the phorophytes, we used the Mann-Whitney U-test. The same statistical analysis was used to evaluate the possible relationship of the height where the individuals of the species are located with the degree of damage.

The annual comparisons of means of the vegetative and reproductive variables were analyzed by one-way ANOVA. To establish similarities or differences between groups, we used the Student-Newman-Keuls.

## Results

*Orchids and the phorophyte community* — In the five 20 × 20 m plots, *B. cubensis* occupied 205 trees and 17 different phorophyte species. The most common host trees were *Plumeria tuberculata* Lodd. (37.1%) and *Erythroxylum areolatum* L. (10.2%). In the three 20 × 20 m *D. lindenii* plots, we found plants on 92 trees representing 13 different species. The most common phorophytes were *Comocladia dentata* Jacq. (16%), *Maba crasinervis* Urb. (13%), and *Cedrela odorata* L. (9%).

*Damage caused by Hurricane Ivan: height and DBH of phorophytes* — The severity of hurricane damage to *B. cubensis* phorophytes was independent of phorophyte height (Mann-Whitney U-test 409.9:  $p=0.336$ ;  $n=121$ ) and DBH (Mann-Whitney U-test 415.5:  $p=0.402$ ;  $n=121$ ). Damage to phorophytes of *D. lindenii* was significantly dependent on tree height (Mann-Whitney U-test 29.0:  $p<0.04$ ;  $n=22$ ), yet severity of damage was independent of DBH (Mann-Whitney U-test 415.5:  $p=0.228$ ;  $n=22$ ).

*Impact of Hurricane Ivan on populations of B. cubensis and D. lindenii* — Prior to Hurricane Ivan the population of *B. cubensis* had 377 individuals in the five plots. The storm reduced the number of individuals by 29.2%. In the following years, the population began to recover but had not yet reached the numbers before the storm. In 2011 and 2012 the population suffered predation by people in plot 3, which negatively affected the trajectory of population growth (Table 1).

TABLE 1. Census totals of *B. cubensis* in 20 × 20 m plots. The 2004 census was made prior to the hurricane.

Plots	2004	2005	2006	2007	2008	2009	2010	2011	2012
1	120	77	88	85	84	81	84	73	66
2	51	36	46	43	41	42	42	45	46
3	72	50	52	55	54	55	45	27	18
4	46	43	52	50	48	50	49	42	41
5	88	77	86	89	93	95	100	137	132
<b>Total</b>	<b>377</b>	<b>283</b>	<b>324</b>	<b>322</b>	<b>320</b>	<b>323</b>	<b>320</b>	<b>324</b>	<b>303</b>

TABLE 2. Census totals of *D. lindenii* in 20 × 20 m plots. Pre-hurricane data are from 2003.

Plots	2003	2004	2005	2006	2007	2008	2009	2010	2011
1	63	30	28	27	24	24	22	19	17
2	45	26	23	20	19	17	13	14	14
3	45	8	7	6	5	2	0	0	0
<b>Total</b>	<b>153</b>	<b>64</b>	<b>58</b>	<b>53</b>	<b>48</b>	<b>43</b>	<b>35</b>	<b>33</b>	<b>31</b>

The population of *D. lindenii* had been much smaller than that of *B. cubensis*. Before the hurricane (2003), we censused 153 individuals in the three plots. Hurricane Ivan immediately reduced the size of the population by 60%, and it has continued to decrease since then (Table 2).

The positional height of *B. cubensis* on their phorophytes had no differential effect on plant damage (Fig. 1), but there is a strong relationship between height above ground and damage to *D. lindenii* (Fig. 2).

*Hurricane impacts on vegetative traits of B. cubensis and D. lindenii* — For this analysis, we used 2006-2012 data from both plots and transects. Prior to the hurricane

in 2004, *B. cubensis* averaged 4.8 leaves per plant. Afterwards, the mean dropped to 2.6, and by 2012 the plants had not yet recovered to pre-hurricane levels (F: 5.982;  $p < 0.0001$ ). While leaf number did not recover, leaf area did to the extent that in 2012 values exceeded previous ones. The values for 2005, 2006, and 2007 were significantly different (F: 3.273;  $p < 0.001$ ) (Table 3). *Dendrophylax lindenii* showed the same behavior as *B. cubensis* (Table 4). Hurricane Ivan caused a loss in number of living roots. With a mean of 8.67 roots per plant, it fell to 3.62. In the following years the species did not recover with highly significant differences (F: 24.438;  $p < 0.0001$ ). In the case of the mean length of the living roots, the same thing happened as in *B. cubensis*. The values for 2004 and 2005 were significantly different each year (F: 12.778;  $p < 0.0001$ ).

*Hurricane impacts on reproductive effort and success for B. cubensis and D. lindenii* — Before Hurricane Ivan, 94.7% of adult plants of *B. cubensis* produced flowers. The hurricane drastically reduced reproductive

TABLE 3. Recovery based on number of leaves and leaf area in *B. cubensis*. The 2004 data are pre-hurricane. Trait values followed by different letters are significantly different from one another.

Years	Number of leaves	Leaf area (cm <sup>2</sup> )	n
2004	4.83 ± 5.05 (a)	15.41 ± 41.28 (ac)	242
2005	2.56 ± 3.12 (b)	6.02 ± 16.75 (b)	162
2006	3.17 ± 3.56 (bc)	7.52 ± 22.5 (b)	461
2007	3.38 ± 3.98 (c)	9.08 ± 31.68 (bc)	463
2008	3.31 ± 3.31 (c)	13.02 ± 47.6 (ac)	458
2009	3.16 ± 4.79 (bc)	14.34 ± 55.52 (ac)	461
2010	3.65 ± 2.42 (c)	13.36 ± 23.62 (ac)	447
2011	3.5 ± 2.96 (c)	14.74 ± 19.97 (ac)	454
2012	3.5 ± 1.48 (c)	16.89 ± 16.18 (c)	427

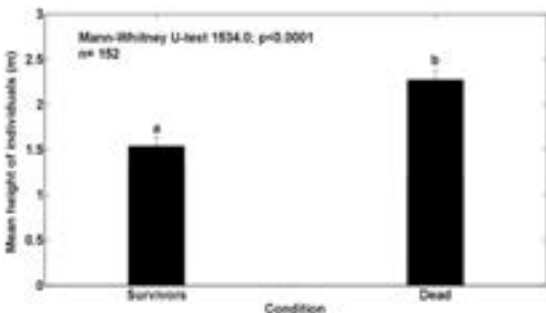


FIGURE 1. Relationship between height on tree and degree of damage to *B. cubensis*.

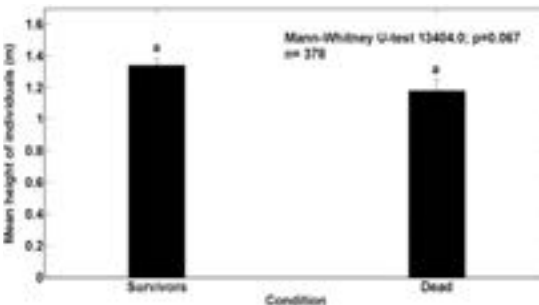


FIGURE 2. Relationship between height on tree and degree of damage to *D. lindenii*.



TABLE 4. Recovery of *D. lindenii* based on root characteristics. The 2003 data are pre-hurricane. Traits values followed by different letters are significantly different from one another.

Years	Number of living roots	Mean length of living roots (cm)	n
2003	8.67 ± 3.54 (a)	10.19 ± 4.88 (a)	153
2004	3.62 ± 3.74 (b)	6.74 ± 5.05 (b)	64
2005	6.51 ± 3.53 (d)	8.67 ± 5.22 (ac)	58
2006	6.45 ± 3.14 (d)	11.88 ± 5.49 (cd)	342
2007	5.74 ± 2.63 (cd)	12.29 ± 6.55 (cd)	304
2008	6.29 ± 2.25 (d)	12.71 ± 6.63 (d)	274
2009	5.87 ± 3.79 (cd)	12.19 ± 8.16 (cd)	301
2010	5.05 ± 2.46 (c)	13.66 ± 8.76 (de)	248
2011	6.21 ± 2.95 (d)	14.83 ± 8.98 (e)	243

effort, and since 2007 recovery has been slow (Fig. 3). Before Hurricane Ivan, nearly a third of adult *D. lindenii* plants produced flowers (Fig. 4). The immediate impact of the storm was dramatic. No plants produced flowers in the first season after the storm. Recovery has been slow, and the last four census years showed stagnation and perhaps even an indication of decline.

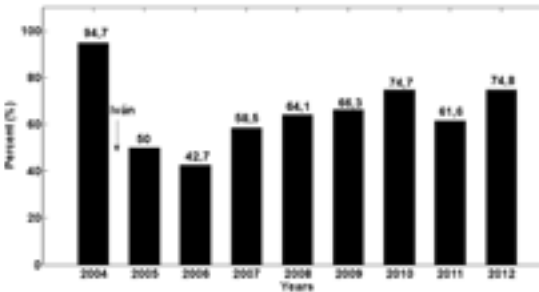


FIGURE 3. Proportion of *B. cubensis* producing flowers annually. The 2004 data were obtained prior to the hurricane.

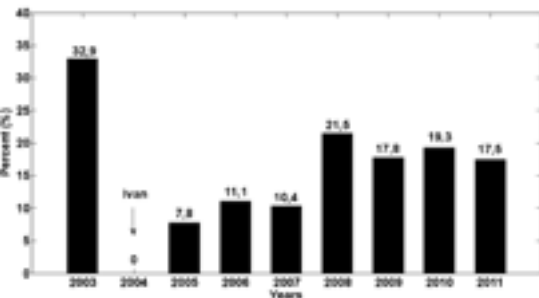


FIGURE 4. Proportion of *D. lindenii* producing flowers annually. The 2003 data were obtained prior to the hurricane.

A year after the hurricane there was a slight, non-significant drop in the length of *B. cubensis* inflorescences (Fig. 5), but subsequently plants of most years (2006, 2008-2012) produced longer inflorescences. In the flowering season after the hurricane, *D. lindenii* failed to produce inflorescences, but in the following year (2005) plants produced robust inflorescences on a par with pre-hurricane production. Unfortunately, this was not sustained, and in all years since then inflorescence lengths were significantly shorter by 1/3 to 1/2 that of 2003 or 2005 (Fig. 6).

The number of flowers per inflorescence produced by *B. cubensis* was reduced after the hurricane but recovered to pre-hurricane levels by 2012 (Fig. 7). The recovery pattern was similar in *D. lindenii*, although no year was significantly different from any other (except the year after the hurricane when no inflorescences were produced). Inflorescences are usually one- or two- flowered, and there is a non-significant trend for more two-flowered inflorescences over the last three years of censuses (Fig. 8).

Whereas reproductive effort improved for *B. cubensis*, reproductive success did not. Fruit set in the

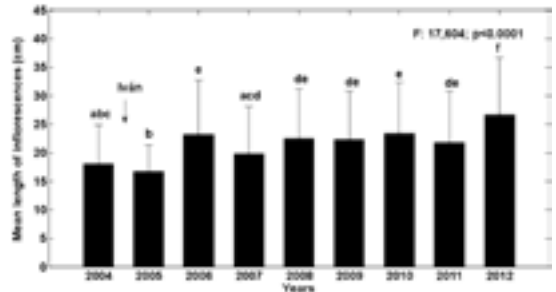


FIGURE 5. Mean length of the inflorescences in *B. cubensis*. The 2004 data were obtained prior to the hurricane.

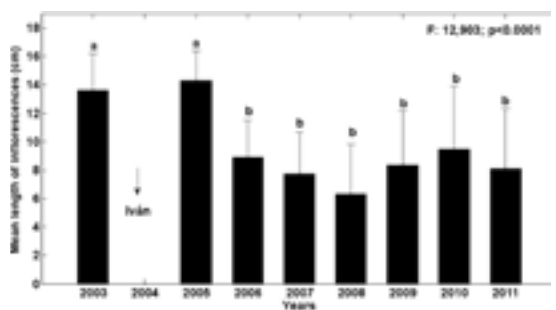


FIGURE 6. Mean length of the inflorescences in *D. lindenii*. The 2003 data were obtained prior to the hurricane.

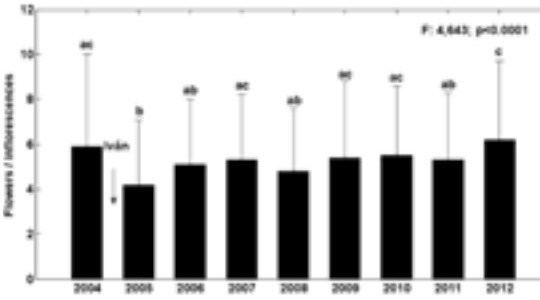


FIGURE 7. Mean number of flowers produced per inflorescence in *B. cubensis*. The 2003 data were obtained prior to the hurricane.

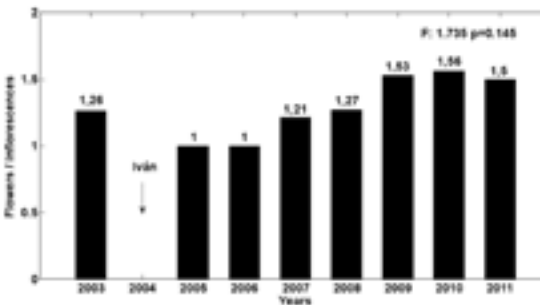


FIGURE 8. Mean number of flowers produced per inflorescence in *D. lindenii*. The 2003 data were obtained prior to the hurricane.

pre-hurricane year was about 21%, but for all post-hurricane years it did not exceed 7% (Table 5). The case of *D. lindenii* is different. Prior to the hurricane there was no fruit set, and occasionally this was repeated through the years. However, sometimes fruit set rose as high as 28% but was not sustained. The number of fruits produced in the population remained low regardless of the number of flowers produced in the population. Pollinator service for *D. lindenii* in the semideciduous forests of Guanahacabibes is low and erratic (Table 6).

The impact of *Hurricane Ivan on demography of B. cubensis and D. lindenii* — The population of *B. cubensis* suffered demographically from the hurricane but recovered a year later and was relatively stable even though one plot suffered predation from plant collectors in the last two censuses (Fig. 9). *Dendrophyllax lindenii* also suffered mortality from the passage of Ivan and for a few years showed signs of modest recovery; however, in recent years the number of individuals has declined (Fig. 10).

We assessed population growth of both species

TABLE 5. Fruit set in *B. cubensis*.

Years	Flowers	Fruits	%
2004	768	163	21.2
2005	205	14	6.9
2006	639	6	0.9
2007	1019	10	0.9
2008	846	11	1.3
2009	1164	45	3.8
2010	1667	60	3.6
2011	1010	14	1.4
2012	1687	52	3

TABLE 6. Fruit set in *D. lindenii*.

Years	Flowers	Fruits	%
2003	43	0	0
2004	0	0	0
2005	4	1	25
2006	18	5	28
2007	23	0	0
2008	46	6	13
2009	59	3	5
2010	60	3	5
2011	43	3	7

TABLE 7. Yearly and average population growth ( $\lambda$ ) of *B. cubensis* based on matrix analyses.

Pairs of years	Lambda ( $\lambda$ )
2006-2007	1.0529
2007-2008	1.0364
2008-2009	0.9673
2009-2010	1.0011
2010-2011	1.0218
2011-2012	0.8953
<b>2006-2012</b>	<b>0.9989</b>

using matrices of plot and transect data since 2006. *Broughtonia cubensis* was dynamic with four of six year-to-year transitions showing population increases. The most recent decrease (2011-2012) was caused by the loss of 53 individuals that suffered predation (Table 7). To reveal the trajectory of population growth we calculated the mean  $\lambda$  from the 2006-2012 censuses for *B. cubensis* and the mean  $\lambda$  from the 2005-2011 censuses for *D. lindenii*. *Broughtonia cubensis* had a mean  $\lambda$  of 1.00, indicating that over this time period

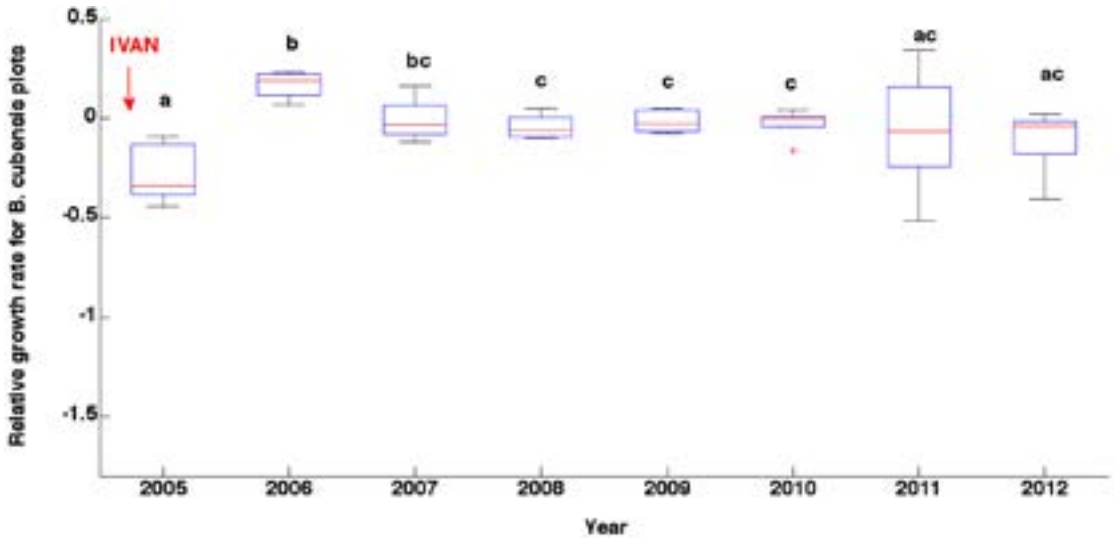


FIGURE 9. Relative population growth rate of *B. cubensis*. This figure is an extended time analysis of Fig. 6 A-B that appeared in *Biotropica* 2013.

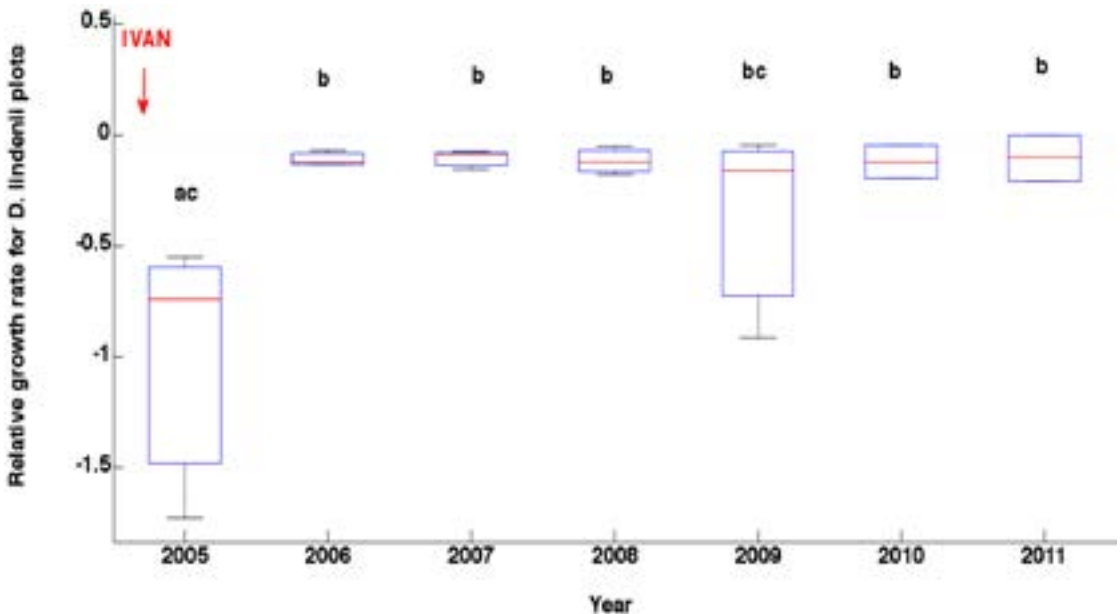


FIGURE 10. Relative population growth rate of *D. lindenii*. This figure is an extended time analysis of Fig. 6 A-B that appeared in *Biotropica* 2013.

the population was stable. The average lambda for *D. lindenii* was 1.05, indicating a slight increase in population size even though most year-to-year transitions were negative. In 2009, seedlings were all found within 90 cm<sup>2</sup> on a single phorophyte causing a high lambda of 1.42 for that year and affecting the overall average.

### Discussion

After the impact of Hurricane Hugo (category 4) in September 18, 1989, on Bisley Watersheds in Puerto Rico, Migenis and Ackerman (1993) observed that the community of epiphytes orchids disappeared. Bayman *et al.* (2002) reported that Hurricane Georges in Puerto Rico produced significant damage in Big

TABLE 8. Yearly and average population growth for *D. lindenii* based on matrix analyses.

Pairs of years	Lambda ( $\lambda$ )
2006-2007	0.9977
2007-2008	0.9171
2008-2009	1.4173
2009-2010	0.8966
2010-2011	0.9624
<b>2006-2011</b>	<b>1.0541</b>

Gulch forest with pronounced negative consequences in the epiphyte community. According to the studies of Tanner *et al.* (1991), the range of damage of a hurricane on a forest varies considerably from country to country. This study demonstrates that all the phorophyte communities located in the same area did not suffer equally.

Generally the studies carried out in the areas impacted by hurricanes show that trees of high DBH rate are more susceptible to being severely affected by the fracture of trunks and branches (Webb 1989; Gresham *et al.* 1991). Bellingham and Tanner (1995), in their studies about the consequences of the impact of the Hurricane Gilbert in September of 1988 in Jamaica, did not find height and DBH of trees severely affected. The height of *B. cubensis* phorophytes was not associated with severity of hurricane damage to those trees. On the other hand, trees with larger diameters suffered more. In the semi-deciduous forest habitat of *D. lindenii*, the tallest phorophytes were more severely affected, and damage class was unrelated to DBH. We conclude that damage severity is both taxon-dependent and habitat-dependent. The same was observed by Walker *et al.* (1992) after the effects of Hurricane Hugo on a Puerto Rican rain forest.

The height to which individual orchids are on the phorophytes did not play any role on the degree of damage by Hurricane Ivan. All orchids have an equal chance of being affected. Six years after Hurricane Ivan, certain vegetative traits of the two species have not yet fully recovered: average number of leaves per individual for *B. cubensis* and the number of live roots of *D. lindenii*. However, average leaf area of *B. cubensis* and average length of live roots of *D. lindenii* were higher six years after the storm than before it. Apparently, investment in leaf (*B. cubensis*) and root

(*D. lindenii*) photosynthetic area has priority over the production of more leaves and roots.

Reproductive effort has yet to fully recover as the percentage of adults producing flowers remained below pre-hurricane levels for both species. The increase of leaf area in *B. cubensis* apparently reflected positively on the average increase in length of the inflorescence. As for the mean number of flowers per inflorescence, both species showed a full recovery. For both species, most worrisome are the low rates of fruit set, apparently caused by the negative impact of the hurricane on the community of pollinators; however, after Hurricane Hugo in Puerto Rico, pollinator activity was higher than in any of the previous years monitored (Ackerman and Moya 1996).

The growth rate of *D. lindenii* since Hurricane Ivan has been continuously decreasing, whereas the gradual recovery of *B. cubensis* has been affected by predation. The matrix analysis has shown that the *B. cubensis* population is stable, but paradoxically the growth trend of *D. lindenii* is generally upward for the transition that occurred in 2008-2009. However, later the species showed a sharp decrease, indicating little possibility of maintaining a superior lambda or close to 1 that allows an upward curve recovery.

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## TROPICAL ORCHID MYCORRHIZAE: POTENTIAL APPLICATIONS IN ORCHID CONSERVATION, COMMERCIALIZATION, AND BEYOND

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**ABSTRACT.** Orchid mycorrhizae are unique interactions in the plant kingdom involving all the orchids and a variety of fungi including *Rhizoctonia*. Orchids are one of the most charismatic plant families and include at least 20,000 species widely appreciated by specialist growers and scientists. They also include *Vanilla*, source of one of the most traded spices worldwide. Most mycorrhizal fungi belong to a group of basidiomycetes widely known for their pathogenic interaction with many crop plants including potatoes, rice, and beans. The main application of orchid mycorrhizal fungi is in the propagation of endangered and commercial orchid species, but we have recently documented an alternative use. The fungal symbionts of orchids have the ability to induce resistance to *Rhizoctonia* in rice plants, which opens new possibilities of biological control agents never previously imagined.

**KEY WORDS:** mycorrhiza, fungi, Orchidaceae, *Rhizoctonia*, biological control

Comprising 10% of angiosperm species, Orchidaceae contains an estimated 25,000 different species (Dressler 1993). Within Ecuador, 3784 orchid species are reported, with 3264 species in neighboring Colombia (Dodson 2003). The high diversity of Orchidaceae in these countries reflects the presence of two biodiversity hotspots in the northwestern region of South America: the tropical Andean region and the Chocó-Darien biodiversity hotspot (Myers *et al.* 2002).

Orchids have attracted considerable scientific interest since Darwin's seminal publication (1877). Unfortunately, the fascinating and diverse floral morphology of orchids has also led in recent decades to serious threats to the survival of many orchid species. Particularly in regions with weak law-enforcement, considerable pressure is exerted on natural orchid populations from unsustainable extraction of specimens from their natural populations for commercialization. The value of ornamental trade in orchids has been estimated at US \$2 billion annually (Harron *et al.* 2007). The ornamental orchid market in Taiwan alone was reported to be valued at US \$114 million in 2012. Although the Taiwan industry is largely based on *Phalaenopsis* species, breeders in

that and other countries grow and export species native to the Neotropics. Orchid commercialization is also a growing industry in Neotropical countries. While many, if not most, orchid commercialization ventures are based on the sustainable propagation of plants *ex situ*, the novelty value of rare endemic species (which are often difficult or impossible to cultivate) means these species are subjected to continuing collection pressures. Additionally, currently non-commercialized species may provide valuable genetic resources in the development of novel commercial hybrids. It is not without reason that the whole of Orchidaceae with the exception of artificially propagated hybrids in the genera *Cymbidium*, *Dendrobium*, *Phalaenopsis*, and *Vanda* has been placed on Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna & Flora (CITES), which lists species that are not necessarily now threatened with extinction but that may become so unless trade is closely controlled.

Compounding the negative impact of wild specimen collection on orchid populations is the fact that many orchid species tend to be rare in the wild, often with small, hyper-dispersed populations. This is particularly true for epiphytic species, which comprise

an estimated 70% of all orchid species (Zotz, in press), with the great majority of these species occurring in tropical regions. In addition to extraction pressures, many orchid populations also face challenges to their survival from habitat destruction and ecosystem degradation, effects that are likely to be exacerbated as a consequence of their specialized interactions with pollinators, phorophytes, and mycorrhizal fungi. Furthermore, a recent study indicates that other, as yet unidentified factors related to microhabitat conditions may be limiting orchid distribution and abundances (McCormick *et al.* 2012). There is clearly much we still have to learn regarding orchid biology in natural habitats, in particular for tropical species, in order to understand the evolutionary processes underlying the high species diversity in this plant family and also identify factors that determine distributions and abundances of orchids in the wild so as to develop effective conservation strategies for them.

The combination of commercial interest in tropical orchids and the rapid rate of ecosystem degradation in tropical regions has led to dramatic declines in many orchid populations (Seaton 2007). Among many conservation measures that need to be implemented, key requisites for ensuring orchid population survival are the maintenance of the levels of recruitment of individuals into established populations as well as the restoration of orchid populations in suitable sites.

Orchid research has principally focused on taxonomy and the role played by pollination biology in diversification (Micheneau *et al.* 2009; Schiestl & Schluter 2009). However, other aspects of their biology, in particular their mycorrhizal interactions, are increasingly gaining prominence in the scientific literature (e.g. Rasmussen 1995; Dearnaley 2007; Kottke & Suarez 2009; Hossain *et al.* 2013). In addition to the specialized relationships orchids often have with their pollinators, accumulating studies have revealed that some epiphytic orchids may also have strong preferences for their host tree (phorophyte) as well as their associated mycorrhizal fungi. Clear examples of orchid distributions being limited with respect to phorophyte have been reported (e.g. Gowland *et al.* 2007, 2013; Crain 2012). Varying degrees of mycorrhizal specificity have been observed throughout Orchidaceae (Kottke *et al.* 2009; Yuang *et al.* 2010; Valadares *et al.* 2012), in particular in achlorophyllous

species (Taylor & Bruns 1997; Selosse & Roy 2009). In tropical taxa, studies of epiphytic orchids in Oncidiinae have also revealed moderate to high levels of preference for specific clades of *Ceratobasidium* spp. (Otero *et al.* 2002, 2004, 2007; Valadares *et al.* 2012). More recently, Martos *et al.* (2012) showed a general pattern for different fungal preferences among orchids with terrestrial and epiphytic habits on the island of Réunion.

Although historically the greater focus has been placed on orchid pollination biology, it is likely that seedling establishment, rather than seed set, is the limiting step in orchid life cycles (Calvo 1993; Otero & Flanagan 2006; Tremblay & Otero 2009). One pollination event will yield millions of seeds that are generally wind-dispersed. Seed germination and seedling establishment, in contrast, may be more challenging for the orchid plant. Lacking endosperm, orchid seeds have minimal energy resources for germination and rely instead upon mycorrhizal symbioses to provide carbon and nutrients (Bidartondo *et al.* 2004; Selosse & Roy 2009). Thus the availability of a suitable mycorrhizal fungus is crucial to orchid establishment in the wild, and studies of orchid mycorrhizae across the family have yielded a complicated panorama of orchid mycorrhizal associations with varying degrees of specificity to one or several different fungal taxa (e.g. Otero *et al.* 2002; Martos *et al.* 2012).

Orchid mycorrhizal interactions also occur in adult plants. In photosynthetic plants it has been generally assumed that the provision of carbon to the plant from the fungus is not essential, yet it seems that mixotrophic nutritional strategies, in which the plant receives carbon from both photosynthesis and mycorrhizal interaction, are common (Selosse & Roy 2009; Roy *et al.* 2013). In this case it may be that different parts of the plant receive carbon provision from both sources. There is some indication that those fungi involved in seed germination are not the same as those that associate with adult plants. In tests of seed germination efficiency of different fungal isolates from adult plants, fungi with provenance from other species were sometimes more efficient (Otero *et al.* 2004; Porras & Bayman, 2007).

Generally, mycorrhizal fungi in orchids belong to the *Rhizoctonia*-like Basidiomycetes (Bayman



& Otero 2006), but recent studies have shown that other groups of fungi can be involved. For example, members of the Basidiomycete 'rust' lineage (Atractiellomycetes, Pucciniomycotina) are mycobionts of orchids (Kottke *et al.* 2009). *Epipactis microphylla* (Ehrh.) Sw. (Neottieae, Orchidaceae) is associated with Ascomycete fungal species allied to the ectomycorrhizal Septomycetes, including truffles (Selosse *et al.* 2004).

*Rhizoctonia*-like fungi may be characterized by certain hyphal morphological traits, including: a lack of conidia; the hyphal branch at a right angle; a septum located a short distance from a constricted ramification; and presence of moniloid cells, a special type of cell with a rounder shape, mainly in sclerotic structures (Roberts 1999). The young hyphae can be multi- or binucleate and rarely produce fruiting bodies in culture, making it difficult to distinguish different species from anamorph (asexual) cultures. The teleomorph (sexual stage) of *Rhizoctonia* can be *Ceratobasidium*, *Thanatephorus* (multinucleate), *Tulasnella* or *Sebacina*, which differ morphologically (Roberts 1999). However, it has proved extremely difficult to promote sexual stages from asexual cultures under laboratory conditions. The *Rhizoctonia*-like fungi are grouped into anastomosis groups (AG) based on their capacity for hyphal fusion. Multinucleate *Rhizoctonia* (*Thanatephorus*) have 13 AG (AG1-AG13), and binucleate *Rhizoctonia* (*Ceratobasidium*, *Tulasnella*, and *Sebacina*) have been divided in 15 groups (AG-A to AG-O) (Sneh *et al.* 1991). Despite these known traits, fine-scale characterization of fungal strains based on morphological characters has been a major obstacle to understanding orchid mycorrhizal interactions.

Over the last decade or so, DNA sequence data have been successfully applied to identify different fungal species from pure fungal cultures or even directly from fungal tissue present in the roots of adult plants (Otero *et al.* 2002, 2004, 2005, 2007, 2011; Pereira *et al.* 2003, 2005, 2009; Suarez *et al.* 2008, 2009; Valadares 2012; Mosquera *et al.* 2013). The most frequent gene region sequenced for the *Rhizoctonia*-like fungi identification has been the internal transcribed spacer (ITS) of ribosomal DNA. In tropical orchids, four separate *Ceratobasidium* clades were reported from epiphytic Oncidiinae in Puerto Rico (Otero *et*

*al.* 2002, 2004, 2005) and Central America (Otero *et al.* 2007). Three further related clades have been found in the Brazilian *Coppensia doniana* (Bateman ex W.H.Baxter) Campacci (Oncidiinae), now correctly known as *Gomesa doniana* (Bateman ex W.H.Baxter) M.W.Chase & N.H.Williams (Valadares *et al.* 2012). Another occurs in *Notylia* (Oncidiinae), *Habenaria* (Orchidinae), *Cranichis* (Cranichidinae), *Vanilla* (Vanillinae), *Epidendrum xanthinum* Lindl. (Laeliinae), *Trizeuxis falcata* Lindl. (Oncidiinae), *Maxillaria* (Maxillariinae), and *Dichaea* (Dichaeinae) (Mosquera *et al.* 2010). These *Ceratobasidium* clades associated with Neotropical orchids are not too distantly related to the fungi that are associated with *Pterostylis* (Pterostylidinae) in temperate southeastern Australia (Otero *et al.* 2011). Fungi belonging to the teleomorph *Tulasnella* have also been isolated from species of Pleurothallidinae (Suarez *et al.* 2006) and Laeliinae (Pereira *et al.* 2001, 2005, 2006; Almeida *et al.* 2007), as well as members of *Vanilla* in Puerto Rico (Porras & Bayman, 2007) and Colombia (Mosquera-Espinosa *et al.*, unpubl.).

These studies represent an important step forward for our understanding of the ecological interactions between tropical orchids and their mycorrhizal fungi at both the seed germination stage and in adults. However, the finding of differing patterns of mycorrhizal associations within the small number of tropical orchid species studied so far indicates the importance of continuing exploration of the diversity and functional preferences of these symbioses across the large number of orchids native to northwestern South America. Clearly, those orchid species facing the greatest conservation threats, either from unsustainable extraction for commercialization or from habitat degradation should be prioritized for orchid mycorrhizal studies. Once the orchid mycorrhizal fungi (OMF) have been characterized, studies can then focus on their application in orchid conservation programs for symbiotic orchid propagation from seed for reintroduction and commercial purposes, as well as for other potential applications which we discuss below.

Commercially, there are two main orchid trades – ornamental species and the cultivation of *Vanilla*. In both trades, symbiotic seed germination could provide considerable benefits. While many species may be

propagated from seed asymbiotically or vegetatively *in vitro*, the presence of fungal mycorrhizae is likely to enhance orchid plant hardening and establishment success in reintroduction programs into the wild. During symbiotic germination trials, fungi that give a large advantage to seedling growth over asymbiotic procedures have been identified. However, this is not always straightforward. As mentioned above, fungi isolated from the adult of the species are not always the most effective at promoting seed germination in the same species (Otero & Bayman 2009).

In Puerto Rico, seeds of both *Tolumnia variegata* (Sw.) Braem and *Ionopsis utricularioides* (Sw.) Lindl. were symbiotically germinated using fungi isolated from adults of the same species (Otero *et al.* 2004, 2005). Similarly, seeds of four groups of species of *Pterostylis* s.l. were symbiotically germinated with their mycorrhizal fungi (Otero *et al.* 2011). The three *Ceratohiza* and uninucleate *Rhizoctonia* anamorphs isolated from *Coppensia doniana* Bateman ex W.H.Baxter Campacci [= *Gomesa doniana* (Bateman ex W. H.Baxter) M.W.Chase & N.H.Williams] were also used successfully in the *in vitro* symbiotic germination of the same species (Valadares *et al.* 2012). However, Porras & Bayman (2007) found that seeds of *Vanilla* species germinated better with a *Ceratobasidium* fungus isolated from *Ionopsis utricularioides* than from *Tulasnella* isolated from adult *Vanilla* plants. These findings indicate that considerable study may be needed in order to identify the most appropriate fungal partners for both orchid conservation and commercialization.

Additionally, biotechnological techniques need to be enhanced to improve efficiency of symbiotic germination and reduce possible contamination with other microorganisms. Bayman (2012) developed a formula for *in-situ* symbiotic propagation using calcium alginate and combining orchid seeds and mycorrhizal fungi for *Epidendrum ibaguense* Kunth to facilitate control of the fungal presence under natural conditions.

Vegetative propagation is almost universally used in *Vanilla* cultivation. However, *Vanilla* crops face serious threats from low genetic diversity, leading to increased sensitivity to crop pathogens; propagation from seed would help to promote genetic diversity. At the Universidad Nacional de Colombia, Palmira

campus, Jazmin Alomia is inducing symbiotic germination of *Vanilla calyculata* Schltr. with a *Rhizoctonia*-like fungus (Alomia *et al.*, unpubl.).

In addition to their functional role as orchid mycorrhizae, some *Rhizoctonia*-like fungi are also recognized as plant pathogenic fungi (Sneh *et al.* 1991; Roberts 1999). *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) is pathogenic in many crop species with a worldwide distribution. It causes damping off in beetroot, potato, beans, soya, and cereals including rice and corn (Sneh *et al.* 1991) among others, including some of the most important crops in tropical regions.

The control of pathogenic *Rhizoctonia*-like fungi generally involves chemical and biological control as well as cultural practices. In recent years the use of biocontrol against pathogenic *Rhizoctonia* fungi of human food products has become more widespread as a means of limiting the use of synthetic agrochemicals that may be detrimental to both consumers and the environment. Ideally, a fungal biocontrol agent should specifically target the pathogenic fungus and not those that may be beneficial, such as mycorrhizal and pest pathogens. There are many examples of potential biological control of pathogenic *Rhizoctonia* using mycopathogenic fungi such as *Trichoderma* sp. and *Chaetomium* sp. (Gao *et al.* 2005). A hypovirulent *Rhizoctonia*-like fungus has been used to induce systemic resistance against pathogenic *Rhizoctonia* (Gressel 2001).

In our studies in Colombia, *Rhizoctonia*-like fungi were isolated from a number of different orchid species and evaluated for biocontrol potential against the pathogenic *Rhizoctonia solani* in rice (Mosquera *et al.* 2010). The orchid mycorrhizal fungi OMF were identified using ITS sequences and found to form four discrete groups. The first included fungi from tropical epiphytic orchids; the second included plant pathogenic *Rhizoctonia* species (*Thanatephorus* spp.) used as a positive control; the third included mycorrhizal fungi from terrestrial orchids; and the final group included mycorrhizal fungi from *Vanilla* species (Mosquera *et al.* 2013).

To evaluate whether the OMF were pathogenic on rice, isolates were inoculated on healthy plants in controlled glasshouse conditions designed to favor the pathogenicity of *Rhizoctonia solani* in rice

(high humidity and temperature). The OMF induced some symptoms of pathogenicity in rice, but the severity was significantly lower than those induced by the positive *Rhizoctonia solani* control isolated from local rice plantations (Mosquera *et al.* 2013). However, when the effect on inducing resistance against pathogenic fungi was evaluated, those healthy rice plants inoculated with OMF two days before inoculation with the pathogenic fungi had significantly fewer pathogenicity symptoms than the control plants inoculated only with the pathogenic *Rhizoctonia solani* but without OMF (Mosquera *et al.* 2013). These findings show that non-pathogenic *Rhizoctonia* species that form mycorrhizae with tropical epiphytic orchids may have potential application as biocontrol agents. Further work is needed to understand the biological mechanism through which this effect is mediated and also to develop the technological application.

### Conclusions

While the application of OMF in orchid propagation for research, conservation, and commercial purposes has been broadly recognized, the true potential of this application is still to be realized in Neotropical regions. Effective application of OMF for conservation and commercial purposes requires a considerable amount of *a priori* study in order to determine which combination of plant species and fungal partner is most effective.

Our studies in Colombia are now showing that OMF are potentially valuable biocontrol agents for important crop pathogens, thus providing another tool for reducing the application of agrochemicals on already sensitive tropical ecosystems. This deserves greater exploration and study over the coming years.

**ACKNOWLEDGMENTS.** We thank DIPAL and Hermes from Universidad Nacional de Colombia for support. ATM was supported by a fellowship from Colciencias “Doctorados Nacionales” 2006.

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## POLLINATION OF SLIPPER ORCHIDS (CYPRIPEDIOIDEAE): A REVIEW

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**ABSTRACT.** Cyripedioideae (five genera; ca. 176 species) are widespread in temperate and tropical Asia and America. About a quarter (42) of the species have been studied to determine their pollinators and/or breeding systems. All flowers are one-way traps pollinated by insects of particular types and size. Slipper orchids are self-compatible and pollinated by deceit. Most *Cypripedium* species are pollinated by bees, whereas some smaller-flowered species are pollinated by flies, and a few are pollinated by both. Most bee-pollinated *Cypripedium* species appear to be generalized food mimics. The fly-pollinated species have evolved diverse pollination systems that utilize a variety of flies as pollen vectors, including fruit flies, dung flies, and a fungal spore-eating hoverfly (Syrphidae). Most species of the tropical Asian *Paphiopedilum* and tropical American *Phragmipedium* are pollinated by hoverflies; flowers of many species in both genera have aphid-like spots that attract gravid female hoverflies that normally lay their eggs in aphid colonies. The more brightly colored *Paphiopedilum micranthum* and *Phragmipedium besseae* are pollinated by Hymenoptera. Autogamy is limited but occurs most frequently in *Phragmipedium* species. About two-thirds of the insect-pollinated slipper orchids (25/37) have evolved highly specialized flowers that are pollinated by a single pollinator or several pollinator species in the same genus. Species belonging to the same taxonomic section usually have the same pollination system. The deceit-pollination system of Cyripedioideae appears to have evolved early in diversification of Orchidaceae..

**KEY WORDS:** *Cypripedium*, *Mexipedium*, *Phragmipedium*, *Paphiopedilum*, *Selenipedium*

### Introduction

Slipper orchids (Orchidaceae: Cyripedioideae) include five genera. Most *Cypripedium* species (52 spp.) are distributed in temperate areas of northeast Asia, North America including Mexico, and Europe (Cribb 1997a; World Checklist of Selected Plant Families 2013). *Paphiopedilum* (92 spp.) occurs primarily in tropical and subtropical southeast Asia (Cribb 1987, 1997a; World Checklist of Selected Plant Families 2013). *Phragmipedium* (26 spp.) species are restricted to tropical/subtropical central and South America (Pridgeon *et al.* 1999, World Checklist of Selected Plant Families 2013); *Selenipedium* (5 spp.) occurs in Panama and northern South America (Pridgeon *et al.* 1999; World Checklist of Selected Plant Families 2013), and the monospecific *Mexipedium xerophyticum* (Soto Arenas, Salazar & Hågsater) V.A. Albert & M.W. Chase is endemic to Mexico (Albert and Chase 1992). Cyripedioideae and Apostasioideae are the oldest subfamilies of Orchidaceae (Dressler 1981; Pridgeon *et al.* 1999).

Slipper orchids live in diverse climatic zones and habitats including subalpine forests (*Cypripedium* in China), cloud forests (*Phragmipedium* in the Andes), temperate forests (*Cypripedium* in North America, northeast Asia, and Europe), tropical and subtropical forests (*Paphiopedilum* in Southeast Asia), bogs (*C. reginae* in North America), and even tundra (*C. passerinum* in North America). Flowers vary greatly in size from the Chinese *C. bardolphianum* with thumbnail-sized flowers to the 12-cm-wide flowers of Peru's *Phragmipedium kovachii* J.T. Atwood, Dalström & Ric.Fernández (Atwood *et al.* 2002).

Of the 176 recognized slipper-orchid species (World Checklist of Selected Plant Families 2013), 42 have been subjects of pollination studies (Table 1). The insect-pollinated species include 22 from *Cypripedium*, 12 from *Paphiopedilum*, and six from *Phragmipedium*. *Selenipedium* and *Mexipedium* are unstudied. Four are autogamous, including the subarctic *C. passerinum* (Catling 1990) and three from *Phragmipedium*: *P. reticulatum*, *P.*

*lindenii* (Pemberton 2011; Koopowitz 2008), and *P. boissertianum* (Pridgeon *et al.* 1999).

### Pollination mechanism

Despite their wide geographical distribution, diverse habitats, and sizes, all species have the same basic floral morphology and pollination mechanism (Cribb 1987, 1997b; Bernhardt and Edens-Meier 2010; Edens-Meier *et al.*, in press). Species are self-compatible but require insects to transfer pollen from the anther to the stigma (Edens-Meier *et al.* 2011). None of their flowers is known to produce nectar or other rewards for pollinators and so are pollinated by deceit (Nilsson 1979; Edens-Meier *et al.*, in press). The lip is expanded into a pouch with a dorsal opening, a distinctive feature that is readily recognized. A small lateral opening on each side of the lip is created by the interlocking bases of the lip and column. The two anthers are positioned on either side of the column inside each lateral opening. Potential pollinators enter the lip's dorsal opening and become trapped inside. The only escape is through one of the lateral openings at the base of the lip. To reach either of these openings, the insect crawls on the interior of the pouch upward toward the stigma, which is positioned with its receptive surface facing the inner surface of the lip. The insect must then squeeze beneath the stigma's receptive surface. If the insect carries pollen, it would be on the dorsal surface of its thorax. Papilli on the stigma scrape the pollen from the insect as it moves beneath. After passing beneath the stigma, the insect reaches one of the anthers at a lateral opening. To escape, the insect must squeeze beneath the anther, receiving a pollen smear or mass applied to its thorax.

In order for a trapped insect to be a pollinator, it must be of a particular size that enables it to squeeze beneath the stigma and the floor of the lip and then beneath the anther and out of through a lateral opening (Bänziger 2005; Li *et al.* 2008a, b). This mechanism assures both pollen delivery and pollen removal. If the insect is too large, which usually means that its thorax is too thick, it cannot escape. Such an insect will usually die inside the lip or become stuck in a lateral opening (Pemberton 2011), but some bumblebees (*Bombus*) are able to escape

through the dorsal opening. If, on the other hand, the insect is too small, it easily passes beneath the stigma and an anther without contacting either, neither delivering nor removing pollen during its escape. Although species are self-compatible, the position of the stigma and anthers and the unidirectional movement of the pollinator beneath the stigma and anther prevent self-pollination. Self-pollination would be possible if the pollinator re-enters a flower previously visited or a different flower on the same plant, but after the pollinator's arduous escape, it usually flies quickly away from the flower and the area. However, individual *Lasioglossum* bees have been recorded re-entering previously visited flowers of *C. henryi* in China (Li *et al.* 2008b).

### *Cypripedium* pollination

*Cypripedium* species diverged earlier than the other genera (Guo *et al.* 2012) and have the greatest diversity of pollination systems and pollinator types. Pollination by large bees, all *Bombus* spp., occurs in five large-flowered *Cypripedium* species (section *Cypripedium*). Four of these species are pollinated by *Bombus* queens (rather than workers). One of these, *C. tibeticum* (Fig. 1), lives at in the high-elevation boreal zone in mountains of southwestern China. Its large purple flowers have a lip with a dorsal opening that appears to be black, and the lip often touches or is close to the ground. Mated queen bumblebees emerge from winter hibernation and search for nest sites, which are usually mouse holes. It is thought that the black dorsal opening of the lip resembles a mouse hole. A queen looking for a nest site enters the lip of the flower, becomes trapped, and pollinates it as it escapes. This hypothesis of nest-hole deceit is supported by the fact that none of the queen pollinators carries any provision for its young (pollen or nectar), which means that it has not yet established a nest and so is still searching for a suitable nest site (Li *et al.* 2006). All but a few *Cypripedium* species pollinated by bees belong to section *Cypripedium*. These orchids are colorful and often have fragrance (Barkman *et al.* 1997), indicating that they are generalized mimics of food-reward flowers.

Bee pollinators of slipper orchids are polylectic, meaning that they seek and gather the pollen of flowers





FIGURE 1. *Cypripedium tibeticum* flowers in China's Sichuan Province in China are pollinated by queen bumblebees searching for nest sites (Li *et al.* 2006). The dark entrance of the lip and the position of the flower near the ground are thought to mimic a mouse hole, a common nest site. The queen enters the lip, becomes trapped, and escapes by squeezing beneath the stigma and then an anther at one the lateral openings at the junction of the bases of the lip and petals, delivering and removing pollen in the process. This trap-lip pollination mechanism occurs in all slipper orchids. Most *Cypripedium* species are pollinated by bees but diverse types of flies have recently been found to be the pollinators of small-flowered species.

belonging to diverse species (Edens-Meier *et al.*, in press), a characteristic that facilitates their attraction and entrapment. Eight *Cypripedium* species are pollinated by small bees belonging to different bee families. The most important bee family is Halictidae (sweat bees), followed by the Andrenidae (mining bees). A few bee species of minor importance belong to the Megachilidae (leaf-cutter bees), Colletidae (plaster bees), and Apidae (varied bees including bumblebees and honeybees). It is notable that honeybee pollination has been detected in only a single species of *Cypripedioideae*, the North American *C. reginae*, which has diverse pollinators; honeybees are not native to North America. Bees in Halictidae and Andrenidae are the sole pollinators of some *Cypripedium* orchids. The halictid pollinators belong to four genera with *Lasioglossum* species being

the most important. *Lasioglossum* bees are the sole pollinators of four *Cypripedium* species, as well as being part of the bee guild pollinators of an additional four *Cypripedium* species. The genus *Lasioglossum* is nearly pandemic (Michener 2000), and species in this genus pollinate *Cypripedium* in Asia, North America, and Europe. *Andrena* is a Holarctic genus with species that pollinate *Cypripedium* in Asia, North America, and Europe (Michener 2000). Sister *Cypripedium* species occurring in China and North America share pollination characteristics. Both *C. flavum* (China) and *C. reginae* (North America), both in section *Cypripedium*, are generalists employing bees and flies as pollinators. *Cypripedium plectrochilum* (China) and *C. arietinum* (North America), both in section *Arietinum*, have similar and unusual floral morphologies, and both are pollinated only by *Lasioglossum* bees.

Wasps are known to pollinate the strange flowers of *C. fasciculatum* (North America; section *Emantiopetalum*) in an odd case of brood-site deception. The small, brown flowers produce a mushroom-like odor that attracts several species of diapiiid wasp parasitoids in the genus *Cinetus* (Ferguson and Donham 1999). This wasp's developmental hosts are larvae of tiny fungus flies that breed in mushrooms. Female wasps apparently become trapped in the flowers while searching for fungus-fly larvae in which to oviposit.

All of the fly-pollinated *Cypripedium* species occur in China. Of these, *C. flavum* is unique in being pollinated by a mix of *Andrena* bee species, bumblebee workers, and blow flies that breed in carrion (Bänziger *et al.* 2005; Zheng *et al.* 2011). The other fly-pollinated *Cypripedium* species are narrow specialists, having highly evolved flowers that mimic the materials in which their pollinators breed. Molecular research indicates that these fly-pollinated, small-flowered orchids are in different sections of the genus (Li *et al.* 2011) and are not sister taxa. *Cypripedium bardolphianum* and *C. micranthum* (section *Sinopedilum*) are pollinated by fruit flies (*Drosophila* spp.) that breed in decaying fruit (Zheng *et al.* 2010). Their flowers are dark and smell of decaying fruit. True pollinaria (instead of soft pollen masses or sticky pollen smears as in most slipper orchids) occur in the flowers of both *C. bardolphianum* and *C. micranthum* (Perner 2009; Li *et al.* 2012).

This is astonishing given that pollinaria were thought to occur only in more advanced orchids (Dressler 1981). As the fruit fly moves beneath an anther, the pollinarium is attached to the dorsal area of its thorax. *Cypripedium sichuanense* (section *Trigonopedia*) is pollinated by a dung fly (Li *et al.* 2012), but why the fly is attracted to the purple, vividly marked flowers is not understood. *Cypripedium fargesii* (section *Trigonopedia*) is pollinated by a hoverfly (also called flowerflies, Syrphidae) in the genus *Cheilosia* that eats and disperses spores of fungi that infect leaves (Ren *et al.* 2011, 2012). The fly is attracted to the large hairy patches on the orchid's leaves that resemble fungal infections. Odor molecules associated with *Cladosporium* fungal cultures have been isolated from the orchid's fragrance. This mimesis of fungus-infected foliage to achieve pollination is one of the most bizarre cases in orchid pollination ecology. Fly pollination in most of these small-flowered *Cypripedium* species has been studied only recently, and such research has broadened our concept of pollination in temperate zone species.

### ***Paphiopedilum* pollination**

*Paphiopedilum* species that have been studied are mostly pollinated by hoverflies (Table 1). The flowers of these species are usually drably colored shades of green, brown, and maroon and are marked with spots, stripes, and hairs (Cribb 1987, 1997a), characteristics that are known to be attractive to flies (van der Cingel 2001). The staminodes of the flowers are often large and yellow, a color that contrasts to the other floral parts (Cribb 1987, 1997a). Furthermore, the staminodes usually have distinctive markings or bumps on their outer faces. The staminode of the Chinese *P. dianthum* bears small, light-colored spots, whereas the staminode in *P. villosum* from Thailand bears a raised wart or bump and a shiny surface (Bänziger 1996). The gravid female hoverfly pollinators appear to interpret these staminodes as aphid colonies, which are sought by these particular hoverfly females to lay eggs in for their aphid-eating larvae. Similarly, a wart might be interpreted by the fly as a single large, female aphid that is starting a new colony. Hoverflies are also attracted to honeydew (Henderson 1982), the sweet exudate produced by



FIGURE 2. *Paphiopedilum dianthum* flower about to be pollinated by a female hoverfly in the genus *Episyrphus* in Guanxi Province in southwestern China (Shi *et al.* 2007). Eleven of the 13 studied *Paphiopedilum* species are pollinated by hoverflies, most of which are gravid females in brood-site pollination.

aphids, which shiny staminodes probably mimic. This mimicry of aphid colonies is so effective that hoverfly eggs have been found on and in the labellum, as seen in *P. rothschildianum* (Atwood 1994). The uniformly dull, light brown or white coloration of the flowers of *P. bellatulum* (section *Brachypetalum*) is different than other hoverfly-pollinated *Paphiopedilum* species, but the flower's dense, contrastingly-colored spotting probably attracts the flies. Some hoverfly-pollinated *Paphiopedilum* species such as *P. dianthum* (Fig. 2) are extreme specialists, being pollinated by a single hoverfly species (an *Episyrphus* species; Shi *et al.* 2007). Others such as *P. hirsutissimum* are specialists but are pollinated by several hoverfly species (Shi *et al.* 2009). Hoverfly-pollinated species belong to four subgenera (*Brachypetalum*, *Polyantha*, *Paphiopedilum*, *Sigmatopetalum*). The only studied *Paphiopedilum* species not exclusively pollinated by hoverflies are *P. micranthum*, which is pollinated by several bumblebees (Edens-Meier *et al.*, in press), and *P. armeniacum*, which is pollinated by several bees and a hoverfly (Liu *et al.* 2005). The colors of the flowers of these species in subgenus *Parvisepalum* are brighter than those of most hoverfly-pollinated *Paphiopedilum* species. *Paphiopedilum armeniacum* is bright yellow, whereas *P. micranthum* usually has a pink or white lip and a yellow staminode that contrasts with the colors of the petals and sepals. Yellow is attractive to bees, and most bee-pollinated flowers are yellow or blue (Paracer 2000). *Paphiopedilum armeniacum* is the

only studied *Paphiopedilum* having a generalized pollination system, with both bees and hoverflies as pollinators. Other brightly colored *Paphiopedilum* species are probably also pollinated by Hymenoptera.

### *Phragmipedium* pollination

Little research has been done on the pollination of *Phragmipedium* species. Three of six examined species have flowers that are completely autogamous (Pridgeon *et al.* 1999; Pemberton 2011). *Phragmipedium lindenii*, the strange lipless species, has triandrous flowers with the extra anther appressed to the stigma. The stigma of *P. reticulatum* flowers is expanded laterally and backward so that it contacts the anthers. *Phragmipedium lindenii* and *P. reticulatum* plants have been observed to set fruit in 100% of their flowers (Pemberton 2011). *Phragmipedium boisserianum* is the third autogamous species, and some autogamy has also been observed in *P. schlimii* (Linden ex Rehb.f.) Rolfe (Pridgeon *et al.* 1999) and *P. longifolium* (Koopowitz 2008).

Three of the four insect-pollinated *Phragmipedium* species are narrow specialists, being pollinated by a single insect species. *Phragmipedium pearcei* and *P. caudatum* are each pollinated by female hoverflies of a single species (Pemberton 2011). *Phragmipedium longifolium* is pollinated by both a female hoverfly and a halictid bee (Dodson 1966; Pemberton 2011), although as previously mentioned some plants of this species are autogamous (Koopowitz 2008). The hoverfly-pollinated species (sections *Himantopetalum*, *Lorifolia*, and *Phragmipedium*) have dull-colored, green-and-brown flowers with aphid-like spots on the infolded surface of the lip above the dorsal opening or around the margin of the opening. I observed *Ocyptamus antiphales* (Walker), the female hoverfly pollinator of *P. pearcei* (Fig. 3), fly directly to these aphid-like spots then ricochet into the dorsal opening of the lip (Pemberton 2011). This brood-site pollination in *Phragmipedium* is remarkably similar to the hoverfly-pollinated *Paphiopedilum* species in Asia.

Flowers of *Phragmipedium besseae* are bright red/orange or scarlet and have a velvety surface. Ever since the sensational discovery of *P. besseae* in 1981 (Koopowitz 2008), there has been curiosity and speculation about what pollinates its red flowers, which



FIGURE 3. *Phragmipedium pearcei* flower being pollinated by a female hoverfly, *Ocyptanthes antiphales*, in Ecuador (Pemberton 2011). Hoverflies appear to be the pollinators of most drably colored *Phragmipedium* species in brood-site pollination. This hoverfly and other hoverfly pollinators of both *Phragmipedium* and *Paphiopedilum* species appear to be attracted to aphid-like spotting on the flowers. These hoverflies lay their eggs in aphid colonies for their aphid-eating larvae. females in brood-site pollination.

are unique in cyripediooid orchids. Hummingbirds have been hypothesized as the pollinators (Atwood *et al.* 2002; Fisher 2011) because they pollinate many red-colored flowers of diverse families in tropical America (Rodriguez-Girones & Santamaria 2004). However, the flowers of *P. besseae* are clearly traps for insect pollinator(s) as in all slipper orchids. In addition to the trap morphology, other features indicate insect pollination. The internal margin of the dorsal entrance to the lip is slippery, which facilitates insect capture. The internal surface of the pouch has a central row of hairs leading to the stigma, serving as a ‘stairway’ for insects. Windows in the upper portion of the lip appear to direct captured insects up and back towards the stigma (Fisher 2011). Hummingbirds are, of course, are too large to be trapped. If they probed the lateral openings, the beak could contact the anthers but would be unlikely to reach the receptive surface of the stigma. Flowers of *P. besseae* are pollinated by an undetermined species of tephritid wasp (Pemberton, unpubl.). Tephritid wasps are parasitoids of subterranean scarab larvae (O’Neill 2001).

Wasp pollination of the red-colored *Phragmipedium besseae* is a paradox because it appears that Hymenoptera as well as most insects (with the exception of butterflies) are visually

insensitive to red and have difficulty distinguishing red flowers from green leaves (Rodríguez-Girones & Santamaria 2004). Red is thought to have evolved in bird-pollinated flowers to make them unapparent to insects that could deplete their nectar (Rodríguez-Girones & Santamaria 2004). There is some yellow coloration on the rim of the dorsal entrance of the lip that might attract Hymenoptera to the trap entrance. *Phragmipedium besseae* belongs to section *Micropetalum* (Koopowitz 2008), which contains species with colorful flowers having velvety surfaces, unlike the green-brown *Phragmipedium* species pollinated by hoverflies. The appearance of these flowers indicates that they, like *P. besseae*, are pollinated by Hymenoptera. The magnificent flowers of the recently discovered *P. kovachii* are probably pollinated by large bees, given their large size, fuchsia-purple coloration, and contrasting white to yellow staminode.

There appears to be convergent evolution in *Phragmipedium* and *Paphiopedilum*. The majority of the species in both genera have evolved brood-site pollination by female hoverflies, with their dull-colored flowers and aphid-like spotting above the dorsal opening. The aphid-like spotting is on the staminode in *Paphiopedilum* species and the folded lip in *Phragmipedium*, but the function appears to be the same in both genera. Pollinators of brightly colored *Phragmipedium* and *Paphiopedilum* have been determined in only one species in each genus, and both are pollinated by Hymenoptera.

### ***Selenipedium* and *Mexipedium***

Pollination of *Selenipedium* species has not been studied in detail. I observed the flowers in a population of *S. aequinoctiale* Garay in Ecuador for three days in 2010. Although fruits were present on some plants, no insect visitors were seen. This was probably due to the variable weather with periods without sun and the presence of only a few short-lived flowers on scattered plants, which made observing more than one or two flowers at a time difficult. Dodson (2003) observed an *Anthophora* bee emerging from a flower of this species, but pollination was apparently not seen or documented (Edens-Meier *et al.*, in press).

Pollinators have also not been observed on flowers

of *Mexipedium*. Some fruits have been seen on plants (Soto *et al.* 1990), but because many flowers did not set fruit, the species is unlikely to be autogamous. The flowers are small, white, some with a pink flush, and velvety, as in those of section *Micropetalum* of *Phragmipedium*. Floral morphology is unlike that of fly-pollinated Cypridioideae, and small bees are probably vectors.

### **Conclusions**

Most cypridioid orchids are specialists, having flowers that evolved to attract either bee or fly pollinators. Phylogenetic relationships are usually predictive of pollination systems; species belonging to the same taxonomic section usually have the same pollination system. Fifteen species are pollinated solely by bees and 16 species solely by flies. Fourteen *Cypridium* species are bee-pollinated, compared to four pollinated by flies. In contrast, ten *Paphiopedilum* species are fly-pollinated, whereas only one is bee-pollinated (Table 1). *Phragmipedium* species appear to be mostly fly-pollinated, but few species have been studied. Five species have generalist pollination systems employing both bees and flies: *Cypridium flavum*, *C. montanum*, *C. reginae*, *Paphiopedilum armeniacum*, and *Phragmipedium longifolium*. Two species, *C. fasciculatum* and *Phragmipedium besseae*, are pollinated by wasps.

Within the bee- or fly-pollinated species, degree of pollinator specialization varies considerably. Narrow specialization, defined here as genus-level specialization (multiple pollinator species in the same genus), occurs in eight species. Extreme specialization, in which a single insect pollinates a species, has evolved in 17 taxa. Most studied species (25 of 42) have highly evolved floral morphologies and attractants that allow a tiny proportion of the potential pollinators in their environments to be effective pollinators.

The success of the trap-lip pollination system in Cypridioideae can be evaluated in terms of reproductive success. Fruit set in *Cypridium* orchids is highly variable even within the same species and the same population during different years. For instance, fruit set in a population of *C. parviflorum* in Quebec, Canada, ranged from 22-75% during a four-year

TABLE 1. Pollinators of Cyripedioideae. Adopted and modified from Edens-Meier *et al.* (in press).

Species	Pollinator types and species	References
<i>Cyripedium acaule</i> Aiton	Bumblebees: <i>Bombus</i> spp. (queens)	Stoutamire 1967; Primack & Stacy 1998; Davis 1986
<i>C. arietinum</i> R.Br.	Bee: <i>Lasioglossum</i> sp.	Stoutamire 1967
<i>C. bardolphianum</i> W.W.Sm. & Farrer	Fruit flies: <i>Drosophila</i> spp.	Zheng <i>et al.</i> 2010
<i>C. calceolus</i> L.	Bees: <i>Andrena</i> spp.; <i>Lasioglossum</i> spp.; <i>Nomada</i> sp.; <i>Colletes</i> sp.	Nilsson 1979; Kull 2008; Antonelli <i>et al.</i> 2009
<i>C. candidum</i> Muhl. ex Willd.	Bees: <i>Andrena</i> sp.; <i>Halictus</i> spp.; <i>Lasioglossum</i> spp.; <i>Augochlorella</i> sp.	Catling & Knerer 1980
<i>C. fargesii</i> Franch.	Hoverfly: <i>Cheilosia</i> sp.	Ren <i>et al.</i> 2011; Ren <i>et al.</i> 2012
<i>C. fasciculatum</i> Kellogg	Wasp parasitoids: <i>Cinetus</i> spp. (females)	Ferguson & Donham 1999
<i>C. flavum</i> P.F.Hunt & Summerh.	Bees: <i>Andrena</i> spp. Bumblebees: <i>Bombus</i> spp. (workers); Blow fly: <i>Calliphora</i> sp.	Bänziger <i>et al.</i> 2008; Zheng <i>et al.</i> 2011
<i>C. guttatum</i> Sw.	Bees: <i>Lasioglossum</i> spp.	Bänziger <i>et al.</i> 2005
<i>C. henryi</i> Rolfe	Bees: <i>Lasioglossum</i> spp.	Li <i>et al.</i> 2008b
<i>C. japonicum</i> Thunb.	Bumblebees: <i>Bombus</i> spp. (queens)	Sun <i>et al.</i> 2009
<i>C. macranthos</i> Sw.	Bumblebees: <i>Bombus</i> spp. (queens)	Sugiura <i>et al.</i> 2002
<i>C. micranthum</i> Franch.	Fruit flies: <i>Drosophila</i> spp. (1?)	Li <i>et al.</i> 2012
<i>C. montanum</i> Thunb.	Bees: <i>Andrena</i> spp.; <i>Ceratina</i> sp.; <i>Halictus</i> sp.; <i>Lasioglossum</i> spp.	Vance, unpubl.
<i>C. parviflorum</i> Salisb.	Bees: miscellaneous, small; <i>Osmia</i> , <i>Andrena</i>	Curtis 1954; Light & MacConaill 2006
<i>C. passerinum</i> Richardson	Autogamous	Catling 1990
<i>C. plectrochilum</i> Franch.	Bees: <i>Lasioglossum</i> spp.; <i>Ceratina</i> sp.	Li <i>et al.</i> 2008a
<i>C. reginae</i> Walter	Bees: <i>Apis</i> sp.; <i>Hoplitis</i> sp.; <i>Megachile</i> sp.; <i>Anthophora</i> ; hoverflies: <i>Syrphus</i> spp.? scarab beetle: <i>Trichiotinus</i> spp.?	Edens-Meier <i>et al.</i> 2011; Vogt 1990
<i>C. sichuanense</i> Perner	Dung fly: <i>Scathophaga</i> spp.	Li <i>et al.</i> 2012
<i>C. smithii</i> Schltr. (= <i>C. callicola</i> Schltr.)	Bumblebees: <i>Bombus</i> spp.	Li & Luo 2009
<i>C. tibeticum</i> King ex Rolfe	Bumblebees: <i>Bombus</i> spp. (queens)	Li <i>et al.</i> 2006
<i>C. yunnanense</i> Franch.	Bee: <i>Lasioglossum</i> sp.	Bänziger <i>et al.</i> 2008
<i>Paphiopedilum armeniacum</i> S.C.Chen & F.Y.Liu	Bees: <i>Lasioglossum</i> sp.; <i>Ceratina</i> sp.; hoverfly: <i>Eristalis</i> sp.	Liu <i>et al.</i> 2005
<i>P. barbigerum</i> Tang & F.T.Wang	Hoverflies: <i>Allograpta</i> sp.; <i>Erisyrphus</i> sp.	Shi <i>et al.</i> 2008
<i>P. bellatulum</i> (Rchb.f.) Stein	Hoverflies: Syrphidae	Bänziger 2002
<i>P. callosum</i> (Rchb.f.) Stein	Hoverflies: Syrphidae	Bänziger 2002
<i>P. charlesworthii</i> (Rolfe) Pfitzer	Hoverflies: Syrphidae	Bänziger 2002
<i>P. dianthum</i> Tang & F.T.Wang	Hoverfly: <i>Episyrphus</i> sp.	Shi <i>et al.</i> 2007
<i>P. hirsutissimum</i> (Lindl. ex Hook.) Stein	Hoverflies: <i>Allobaccha</i> sp.; <i>Episyrphus</i> sp.	Shi <i>et al.</i> 2009
<i>P. parishii</i> (Rchb.f.) Stein	Hoverfly: <i>Allograpta</i> sp.	Bänziger 2002
<i>P. micranthum</i> Tang & F.T.Wang	Bumblebees	Edens-Meier <i>et al.</i> (in press)
<i>P. purpuratum</i> (Lindl.) Stein	Hoverfly: <i>Ischiodon</i> sp.	Liu <i>et al.</i> 2004
<i>P. rothschildianum</i> (Rchb.f.) Stein	Hoverfly: <i>Dideopsis</i> sp.	Atwood 1994
<i>P. villosum</i> (Lindl.) Stein	Hoverflies: <i>Betasyrphus</i> sp.; <i>Episyrphus</i> sp.; <i>Syrphus</i> sp.	Bänziger 1996
<i>Phragmipedium besseae</i> Dodson & J.Kuhn	Wasp parasitoid: Tiphidae sp.	Pemberton, unpubl.
<i>P. boisserianum</i> (Rchb.f. & Warsz.) Rolfe	Autogamous	Pridgeon <i>et al.</i> 1999
<i>P. caudatum</i> (Lindl.) Rolfe	Hoverfly: <i>Syrphus</i> sp.	McCook 1989; Pemberton 2011
<i>P. lindenii</i> (Lindl.) Dressler & N.H.Williams	Autogamous	Pemberton 2011
<i>P. longifolium</i> (Warsz. & Rchb.f.) Rolfe	Bee: <i>Chlerogella</i> sp.; Hoverfly: <i>Syrphus</i> sp.	Dodson 1966 Pemberton 2011
<i>P. pearcei</i> (Veitch ex J.Dix) Rauh & Senghas	Hoverfly: <i>Ocyrtamus</i> sp.	Pemberton 2011
<i>P. reticulatum</i> (Rchb.f.) Schltr.	Autogamous	Pemberton 2011

rarely set fruit (Primack & Stacy 1998). Rewardless orchids usually have lower levels of fruit set than do orchids with rewards (Tremblay *et al.* 2005). Fruit set in Cyripedioideae, as in many other long-lived orchids, is often low, but the large numbers of seed per capsule and the longevity of the plants probably compensates. The trap-lip pollination system that efficiently removes and places pollen on captured and escaping insects appears to be successful given the persistence of Cyripedioideae through time and their wide radiation. Much of this success is probably due to the evolution of specific flower forms, coloration, and odors that enable them to attract and use specific insect species or types as pollinators in diverse habitats and climatic zones.

The pollination ecology of the majority of this subfamily (ca. 120 species) is unknown. Recent studies of small-flowered Chinese *Cypripedium* species and Ecuadorian *Phragmipedium* species have led to the discovery of unique and unexpected pollinator types representing novel pollination systems. Additional research may well lead to others.

ACKNOWLEDGMENTS. I thank Alec Pridgeon and Pepe Portilla for inviting me to the 2012 Andean Orchid Symposium where this paper was first presented. Pepe Portilla and the Ecuagenera staff facilitated field work on *Phragmipedium* in Ecuador. Harry Zelenko provided helpful information on *Selenipedium aequinoctiale* also in Ecuador. Luo Yi-bo, Li Peng, and Holger Perner helped with my visits and research on *Cypripedium* in China. Marilyn Light provided valuable information on *C. parviflorum*. Colleagues Retha Edens-Meier, Peter Bernhardt, Luo Yi-bo, and the author extracted the literature to create Table 1, in part; a different and more detailed version will be published elsewhere (Edens-Meier *et al.*, in press). Mark Whitten and Alec Pridgeon provided valuable reviews and edits of the manuscript.

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## TOWARD A GLOBAL ORCHID TAXONOMIC NETWORK

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**ABSTRACT.** The genesis and early establishment of the EPIDENDRA project are reviewed, retracing the scientific, ethical and political reasons that defined its actual shape and contents. The taxonomic structure of the global orchid network, its systematic framework and nomenclatural implications are discussed. The actual figures of the database are presented, and the lines of its future development are pointed out.

**RESUMEN:** Se revisan la génesis y las etapas iniciales del desarrollo del proyecto EPIDENDRA, recapitulando las razones científicas, éticas y políticas que contribuyeron a definir su forma y contenidos actuales. Se discuten la estructura taxonómica de la red global de orquídeas, su marco sistemático e implicaciones nomenclatoriales. Se presentan los hechos y números actuales de la base de datos y se esbozan las líneas de su desarrollo futuro.

**KEY WORDS:** databases, Orchidaceae, botanical nomenclature, EPIDENDRA, history of science

*“S’il publie lui-même ses Orchidées, la moitié sera déjà connue [...]. On sait qu’il set totalement impossible de produire hors de l’Europe un travail parfait [...]. Veuillez exposer ces considérations à votre ami et lui dire que je lui offre de publier ses nouveautés sous notre mutuelle autorité.”*

“If he would publish his orchid species by himself, half of them would be already known [...]. Anybody knows that it is impossible to produce a perfect work outside Europe [...]. Tell to your friend that I offer him to publish his novelties under our shared authority.”

Letter by H. G. Reichenbach  
intended for J. Barbosa Rodrigues  
March 1877

*Apologia pro opere meo.* Looking back to the discussions that led to the original conception and design of EPIDENDRA, the global taxonomic network created and maintained by the Lankester Botanical Garden (LBG) at the University of Costa Rica (UCR), it is hard to believe that more than ten years have elapsed. It is now time to try explaining the genesis of this project and the main forces that molded it into its actual shape and contents and to point out the lines of its future development.

Basically meant to respond the needs of the orchid researchers working at LBG as a sort of digital surrogate of a documentary archive, EPIDENDRA incorporated scientific, ethical and political *reasons* that were not apparent – or at least not deliberately planned – at the beginning. The reconstruction of these reasons and the general significance of EPIDENDRA, as I present it below, are therefore the products of a largely *a posteriori* consciousness. Notwithstanding its imperfections and partiality, the actual structure of the global orchid taxonomic network reflects a scope that goes beyond the simple organization of botanical information as it was originally conceived to convert it into a scientific tool that helps tens of orchid students around the world every day to improve the biological inventory they are committed to carry out. When we look at today’s figures of EPIDENDRA’s on-line visitation, it is noteworthy that the databases are mostly accessed by students working in Neotropical countries, reflecting not only the actual focus of the site requests for taxonomy of tropical American orchids but also the progressive satisfaction of information requirements by local scientific communities. As one of the conceptual authors and a worker of EPIDENDRA, I am not in the position of judging the magnitude of the results, but I can at least relate where we are along this process and why we are there.

### An exemplary story

In 1840, as a worldwide recognized specialist in Brazilian botany, Carl Friedrich Philipp von Martius began editing *Flora Brasiliensis*, a monumental work for which he sought the assistance of the most distinguished botanists of the time to write monographic treatments of various families. When the work was eventually concluded by Ignacio Urban in 1906, 65 naturalists had contributed to the *Flora*: 38 German botanists, five Austrians, five British, five Swiss, four French, two Belgians, two Danes, two Czechoslovaks, a Dutch and a Hungarian (Mello Leitão 1937). None of the contributors was a Brazilian.

Concerning orchids, however, Brazil had then a distinguished student, João Barbosa Rodrigues (Fig. 1A), who in 1870 had presented a work in three volumes with descriptions in Latin and French of hundreds of new orchid species. A copy of the still unpublished work was sent in 1871 to August Endlicher – Martius' successor as the main editor of the *Flora Brasiliensis* –

who, in turn, showed Barbosa Rodrigues' manuscript to Heinrich Gustav Reichenbach (Fig. 1B), the botanist in charge of Orchidaceae. It was obvious that a reasonable treatment of the orchid flora of Brazil was impossible without the specimens and field notes by Barbosa Rodrigues, and Reichenbach tried to obtain them. When in 1877 Barbosa Rodrigues eventually sent to press his "*Genera et species orchidearum novarum*", he published the extract of a letter by Reichenbach directed to his friend Anders Fredrik Regnell (Barbosa Rodrigues 1877). With a tone that one could see as coldly cynical – or crudely honest – the German professor asked Regnell to convince Barbosa Rodrigues to give up its publication, adducing the impossibility for a Brazilian botanist to publish an accurate work without access to the types and literature only available in Europe (Fig. 2). Reichenbach's crude realism illustrates a scientific situation common during the nineteenth century – general aversion to having scientific efforts carried out locally (Neiva 1929; Sá 2001).



FIGURE 1. Two great orchidologists. A — The Brazilian João Barbosa Rodrigues (1842–1909), lately Director of the Rio de Janeiro Botanical Garden. B — The German Heinrich Gustav Reichenbach (1823–1889), Professor of botany and Director of the botanical gardens at Hamburg University.



FIGURE 2. Extract from the letter by Reichenbach to Regnell, dated 22 March 1877 (from Barbosa Rodrigues 1877).

It is a fact that the essential information on the identity of the organisms native to the biologically richest regions of the planet was mostly stored in Europe, and Reichenbach himself (cited in Barbosa Rodrigues 1877) considered as the only exception to this rule the herbarium of North American plants gathered at Harvard by Professor Asa Gray. Study of the biological diversity in the tropical regions has been traditionally hampered by the lack of an appropriate information system – including plant museums and modern herbaria – and the absence of significant historical libraries. This has been an impediment to a vaster documentation of biological variation, which is required for a full understanding of living diversity, ecosystem dynamics and their conservation.

If one looks at the botanical institutions that played an important role in global documentation of plant diversity until the end of nineteenth century, it is easy to see that they were almost exclusively located in those European countries that had a significant colonial history (Fig. 3A) as a result of the scientific and economic interests they had in the exploration

and exploitation of their overseas possessions. Half a century later, botanical predominance spread across the Atlantic to include several distinguished institutions in North America and a few other research centers across the world, mostly in the cultural areas where English was spoken. A simple view of the “world map of botanical excellence” in the first half of the twentieth century (Fig. 3B) clearly reveals that the countries lying in the tropical regions of the Earth, which we could define as “culturally young” areas, are completely absent from this scene. Until the last decades of the second millennium, botany in general, and more specifically the building and maintenance of large repertoires of specimens, books and other materials necessary for the interpretation of plant diversity, largely remained a business of developed countries.

Of the almost 400,000 plants species recorded on Earth, three quarters live in the tropical regions, where rain forests support the greatest diversity of living organisms and the inventory of orchid diversity is incomplete. The paradox, however, is that while a better

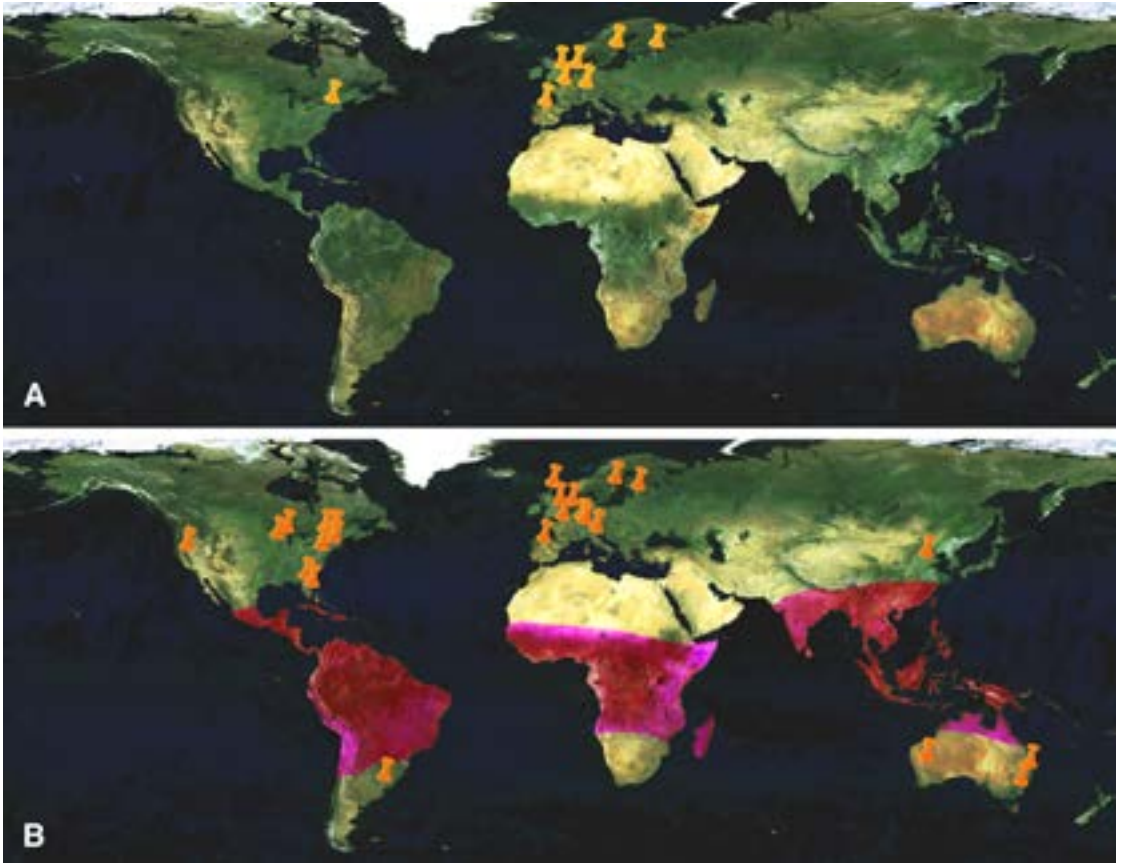


FIGURE 3. Important centers for plants documentation of global interest. A – During the second half of nineteenth century. B – In the first decades of twentieth century. The red areas on the map correspond to regions with tropical vegetation.

documentation system is needed for the identification, comparison and management of the much more diverse tropical floras, and the need for floristic research in the tropics is greater than at any other time in modern history, most of the essential documents and the globally important collections necessary for the interpretation of floristic mega-diversity are stored in developed countries of the temperate regions (Fig. 4). The history of orchidology in Costa Rica is a perfect example of this paradox.

#### Naturalists and botanists

Costa Rica is well known as one of the richest regions in the world in plant species and – at least compared to other floristically diverse areas – a well botanized country. Knowledge of the Costa Rican flora was largely attributable to the continuous presence of a varied group of resident naturalists who carried out

botanical exploration and collection since the second half of the nineteenth century. The list of naturalists who lived in Costa Rica begins with the Germans Carl Hoffmann (1833–1859) and Alexander von Frantzius (1821–1877), followed by Auguste R. Endrés (1838–1875), French by birth but German by culture, and the Swiss Richard Pfau (-1897), who established an orchid firm in San José around 1870. During the 1870s the Swiss Henri Francois Pittier (1857–1950) and Pablo Billely (1861–1908) came to Costa Rica, who were soon joined at the Instituto Físico-Geográfico and the Costa Rican Museo Nacional by Adolphe Tonduz (1862–1921), another Swiss, the German brothers Alexander Curt (1881–1971) and Alfred Brade (1867–1955), the Alsatian Carl Wercklé (1860–1924), and the Costa Ricans Anastasio Alfaro (1865–1951), Alberto Manuel Brenes (1870–1948) (Fig. 5A), and the young Otón Jiménez (1895–1988) (Fig. 5B). Together

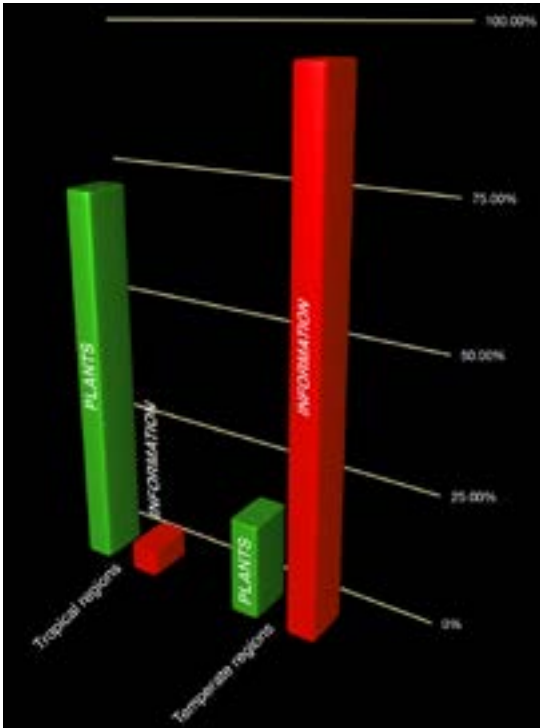


FIGURE 4. Number of plant species versus number of stored type specimens in tropical and developed countries.

they formed a herbarium that, at the beginning of the twentieth century, “was unequalled below the Río Grande del Norte” (Standley 1937). Under the unselfish patronage by Amparo López-Calleja de Zeledón (1870–1951), most of them also contributed specimens to be sent for determination in Europe. Then, during the first decades on twentieth century, the list includes the British Charles Herbert Lankester (1879–1969) (Fig. 5C), his good friend and companion in botanical excursions, the Costa Rican Alfredo Sancho (1876–1929), and Juvenal Valerio Rodríguez (1900–1971) (Fig. 5D), who eventually became one of the founders of the Pan-American Agricultural School “El Zamorano” in Honduras (for a review of orchid-related activities in Costa Rica until the mid-1900s, see Ossenbach 2009).

When studying the orchid specimens gathered by this trained group of individuals, it is evident that they were not collected randomly. They knew the orchid flora of Costa Rica, and selectively prepared specimens that were mostly unknown by science. Of the 165 orchid specimens collected by Wercklé and

cited by Schlechter (1923) in his work on the orchid flora of Costa Rica, more than half (84) were new to science at the time of their discovery (Pupulin 2010). In the same work, Schlechter described 92 new species from the collections of Brenes alone (Barringer 1986). Among the orchid specimens sent by Lankester to his correspondents in Europe and in the United States, 113 were described as new species (Pupulin & Romero 2003). Considering that not a single orchid species from Costa Rica bears the authorship of Biolley, the Brade brothers, Brenes, Jiménez, Lankester, Pittier, Sancho, Tonduz, Valerio, and Wercklé, it is perhaps obvious to ask why they did not make the next logical step in botanical research – naming and describing their orchid collections. The answer is probably simple: they did not have access to types and other critical specimens, original literature and illustrations, etc. with which to compare their new findings. They had no other choice but to form small “scientific societies” with botanists of the developed world, who took charge of the scientific descriptions of the orchid flora of Costa Rica. Heinrich Gustav Reichenbach (Fig. 6A), Robert Allen Rolfe (Fig. 6B), Rudolf Schlechter (Fig. 6C), Oakes Ames (Fig. 6D) and Fritz Kränzlin permanently bound their names to the orchids of Costa Rica. It was inevitable that, within the framework of these societies, the role of the resident naturalists would be mostly interpreted — and sometimes bitterly perceived — as that of “plants providers” or, simply, collectors. “*I begin to consider these cabinet-celebrities as vampires nourishing their inflated fame at the cost of the lifeblood of those poor fools they condescendingly call ‘collectors’*”: these words were written in 1874 by A. R. Endrés, probably the greatest orchidologist who ever worked in Costa Rica (Ossenbach *et al.*, in press).

Things were not substantially different in Costa Rica at the end of the millennium. The research carried out by Dora Emilia Mora-Retana, the first national academic orchidologist and director of LBG for over 25 years, was mostly done in cooperation with foreign students, notably John T. Atwood, who through his activity as the director of the Orchid Identification Center at the Marie Selby Botanical Gardens had access to modern herbaria and library facilities. Their common work culminated in the publication of the Maxillariine and Oncidiinae treatments for the *Flora Costaricensis* (Atwood & Mora-Retana 1999), the results of an advantageous and



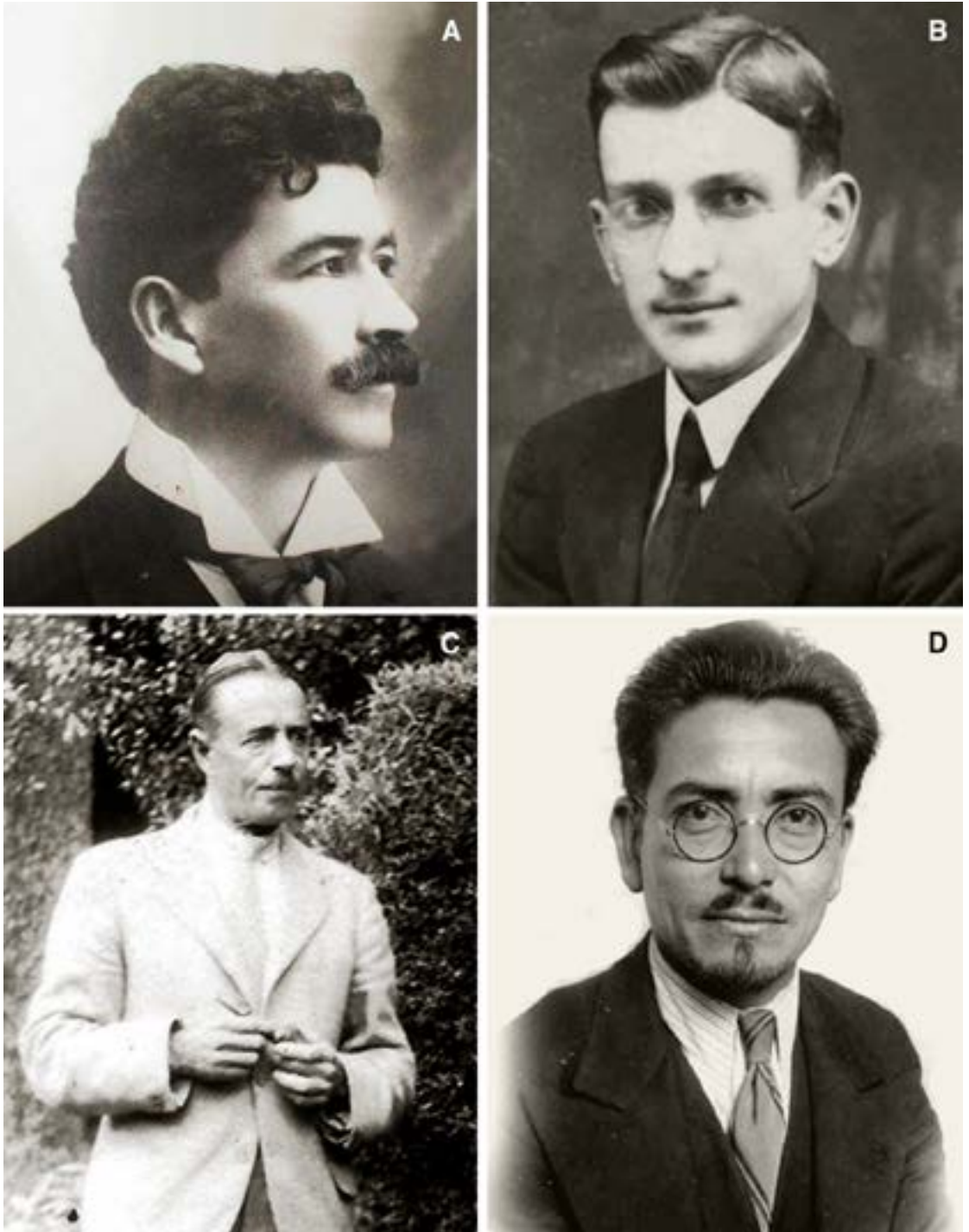


FIGURE 5. Naturalists who collected new orchid species in Costa Rica. A – Alberto M. Brenes. B – Otón Jiménez. C – Charles H. Lankester. D – Juvenal Valerio.

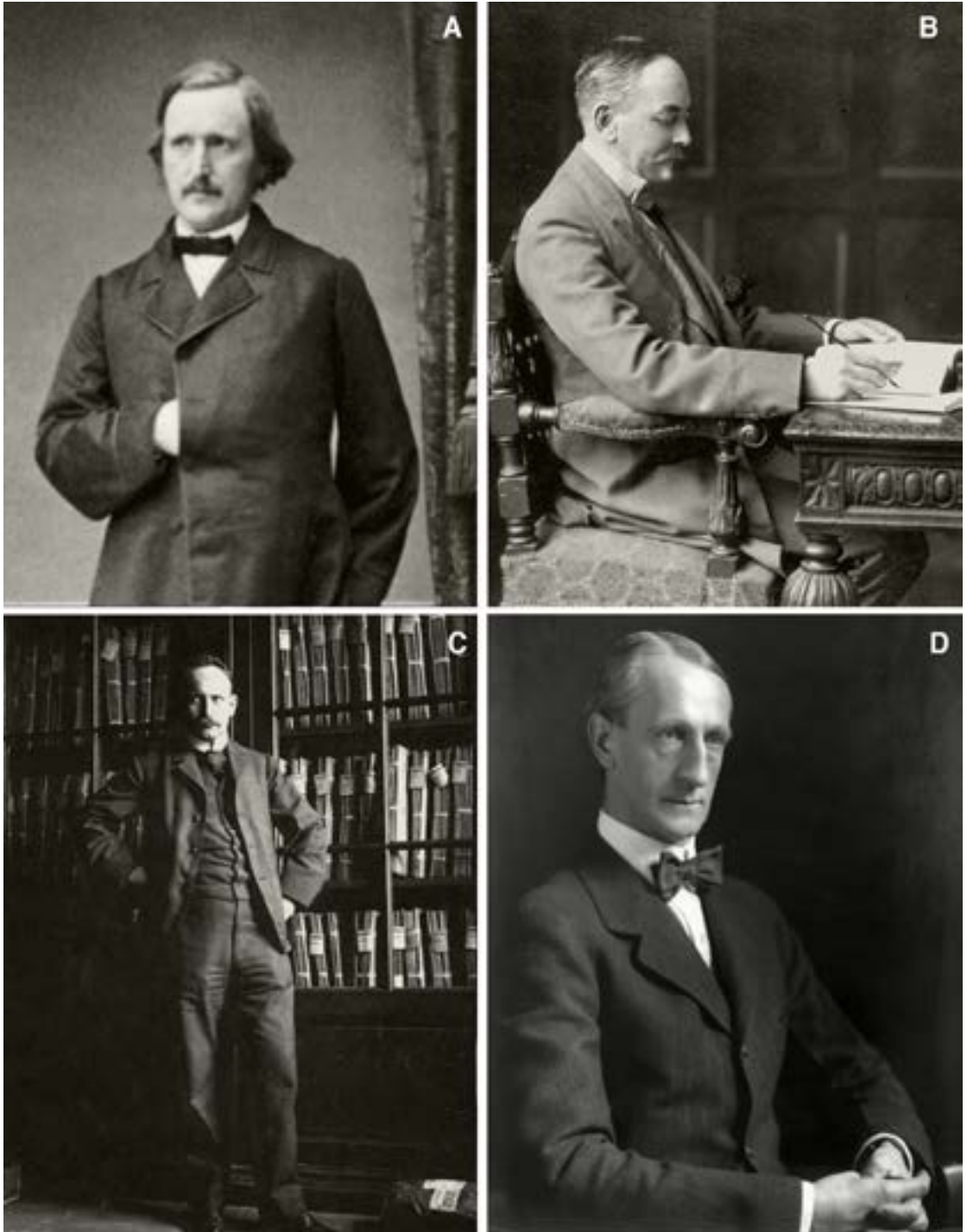


FIGURE 6. Botanists who described new orchid species from Costa Rica. A – Heinrich Gustav Reichenbach. B – Robert Allen Rolfe. C – Rudolf Schlechter. D – Oakes Ames.

necessary cooperation, as Mora-Retana's observations relied on the scrutiny of materials and information not available in Costa Rica.

When, in 2001, I was hired to work as an orchid researcher at LBG, the taxonomic archives of the center were still limited to two file cabinets, and there was no specialized library. Although the scarcity of economic resources has been mostly identified as the principal factor limiting the growth of the biological sciences in tropical countries, we discovered instead that the major limiting factor was the scarcity of information (Pupulin & Warner 2005).

### The advent of the Internet

Images of herbarium specimens in the form of photographs, slides, photocopies, etc., with a special emphasis on nomenclatural types have long been used as complementary materials for taxonomic studies. Early examples of extensive image collections of type specimens date back to the 1930s (i.e., the photographs of European types taken by J. Francis Macbride, now available from the web site of the Field Museum in Chicago), but it was not until the advent of digital data capture in the last two decades that the information sources represented by biological collections kept in developed countries began to be effectively disseminated. Toward this end the project CROTYPES, a joint effort by the LBG and the Oakes Ames Orchid Herbarium, digitized the nearly 800 type sheets of Costa Rican Orchidaceae, probably the richest repository in the world (Pupulin & Romero 2003). Analogous projects were carried out in subsequent years by LBG researchers at the herbaria of the Marie Selby Botanical Gardens, the Royal Botanic Gardens, Kew, the Real Jardín Botánico of Madrid, the Linnean Society of London and the Natural History Museum in Vienna. Rare-to-find, important texts for the interpretation of the orchid flora of Costa Rica were also digitized in the last ten years.

It was a fortunate circumstance that, when the structure of EPIDENDRA was originally discussed, the use of the Internet was already established as the main electronic platform for data interchange. Since 1982 the Missouri Botanical Garden had established with Tropicos its primary supporting database for botanical taxonomic research, with an Internet page that today provides open worldwide access to over 1.2 million

plant names (with synonymy, types, distributions, references, and cross-referenced specimen records; Tropicos 2012). In the mid-1980s the production of an electronic version of *Index Kewensis* gave rise to the International Plant Names Index (IPNI), a database of the names and associated basic bibliographical details of seed plants, ferns and fern allies, a collaborative effort among the Royal Botanic Gardens, Kew, Harvard University Herbaria and the Australian National Herbarium. Currently, it holds more than 1.6 million name citations, 42,000 authors and almost 17,000 publications (IPNI 2012). Later, Rudolf Jenny made public his personal orchid literature database, BIBLIORCHIDEA, which contains most of the existing journal articles, books and preprints on Orchidaceae. With its 150,000 entries, it is maintained today by the Swiss Orchid Foundation at the University of Basel, which also added the World Orchid Iconography, including almost 80,000 records for over 10,000 orchid species. Taken five years to complete, the Royal Botanic Gardens, Kew, published in 2006 the World Checklist of Selected Plant Families as a searchable electronic database consolidating over 200,000 names. The project has now become an international collaborative program with more than 150 contributors throughout the world who update nomenclature and identification of the species (WCSP 2012). Several other databases arose in the last decade, some of them specifically devoted to Orchidaceae, such as the Internet Orchid Species Photo Encyclopedia compiled by Jay Pfahl, the Orchid Picture Reference Database created by the London Orchid Society (Ontario, Canada) with almost 140,000 links to pictures found in over 1500 picture sources. Others have a more regional approach (such as Orchidaceae from Central Africa, including scientific names, distribution data, images, identification keys, links and references). Still others focus on genera, such as Nina Rach's The *Sobralia* Pages and The *Stanhopea* Pages, with images, and information on habitat and culture.

Until recent years, one common characteristic of nomenclatural databases was that they were mainly built as a reference system designed to provide references to plant names, basionyms and synonyms, nomenclatural types and lists of *exsiccata* for selected regions, allowing botanists to gain ready access to the authors of names, titles of key publications





FIGURE 7. A screenshot of a page from the first version of EPIDENDRA website.

and, indirectly, to locations of type specimens. The users, however, are referred to physical documents (protologues and other critical publication, types and other materials associated with the types) that are not available electronically. In this sense, the electronic tools for the retrieval of botanical information presuppose direct access to the sources through libraries and herbaria services. This is often not the case in tropical countries, where facilities are often insufficient and where the lack of historical libraries and the relatively modernity of the existing herbaria represent a major obstacle for botanic research when concerned with the retrieval of historical information (Gómez-Pompa & Nevling 1988; Pupulin & Warner 2005; Pupulin 2007).

In 2002, the conceptual discussion about the characteristic of JBL databases was substantially concluded, and the intranet version of EPIDENDRA began its life. The name of the database, the plural of *Epidendrum*, was an allusion to the old name under which Linnaeus knew all the epiphytic orchids from the tropics. As a mainly internal research

tool, EPIDENDRA was designed to gather and make electronically available all the materials from the garden's collections: the documentation center, the library, the living plant collections and the ancillary collections (spirit, pollinaria, slides, scans, material in silica). When EPIDENDRA eventually opened to the public with the launch of its first web page in 2003 (Fig. 7), the system focused on the direct availability of the original sources as its main attraction (Schug 2003). Since then, the database has been profoundly modified to satisfy the needs of a more demanding public and simplify its access and use, but the conceptual structure of EPIDENDRA has remained almost unchanged until today.

#### EPIDENDRA's taxonomy

In the past, the debate on biological databases mainly focused on the best model to be used in organizing taxonomic data from literature and other sources to avoid oversimplification and reflect the elasticity of taxonomy as well as alternative taxonomies (see, e.g., Berendsohn 1997; Conn 2003).

As taxonomic information may become rapidly outdated in the tropics, we hoped to be able to build a system designed to reduce taxonomic decisions in the database to a minimum as an alternative to the necessity to train specialized staff and thereby increase the cost of the effort.

As botanical names of any rank, independent of their priority and meaning, are equivalent in weight, we visualized a horizontal structure wherein all species names were equally considered: names were simply treated as equivalent doors to gain access to relevant taxonomic information. Our intention was to provide unrestricted access to documentary sources, and a simple but accurate system of cross-referenced synonyms seemed to be the best way to guarantee the easiest access and avoid difficult (and sometimes useless) taxonomic controversies. According to this plan, we were confident that the taxonomic work and therefore the need of specific skills and expertise could be basically reduced to verification of heterotypic synonymies recorded in taxonomic literature through a careful study of original type materials (Fig. 8A). Our plan soon proved to be too optimistic.

First, taxonomic literature is plagued with errors. Species names can be ineffective, illegitimate, invalid or simply inexistent; the simple priority rule in the application of names has often been ignored or distorted, mainly when old and obscure scientific names are concerned; spelling is frequently incorrect; and old and new homonyms — to be disentangled — pop up with unexpected frequency. The uncritical “horizontality” of our system, which should have allowed a sort of automatic implementation of the database, became a taxonomic nightmare. This also applies to supposed automatic, homotypic synonymy: irrespective of accepted generic circumscriptions or *before* such considerations, can generic names confidently be used according to the provisions of the Code? Are they effective, legitimate, valid?

Furthermore, with the inclusion in the database of flower photographs, as well as historical and modern botanical illustrations and drawings, PDF files of relevant literature, and images of the pollinaria, we met another conceptual difficulty. Synonymous names have no reality as living organisms: flowers, pollinaria, and even preserved genetic samples only belong to good species, associated with a correct and accepted name.

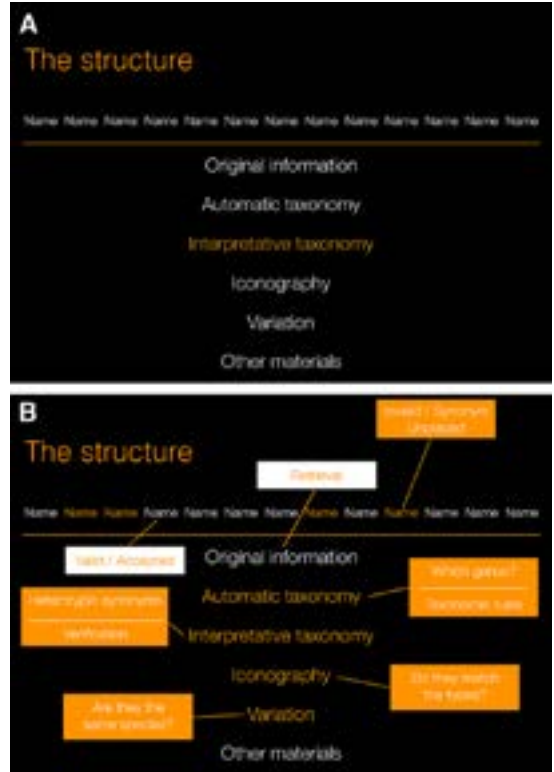


FIGURE 8. The conceptual structure of EPIDENDRA’s taxonomy. A – The originally expected, horizontal, mostly uncritical model. B – The actual, verified model. Orange boxes and letters indicate areas that are dependent on taxonomic decisions.

One step beyond the index, the individual species-files of EPIDENDRA must belong to one of two categories: an accepted name (including all the ancillary iconographic materials) or a synonym, which is just a nomenclatural door to be referred to the accepted name.

It is now quite clear for us that, to maintain an updated and critically evaluated taxonomy and relative taxonomic history, the adoption of one or more alternative taxonomies cannot be avoided, even when the taxonomic system is unofficial (Fig. 8B).

**The systematic framework**

Again, EPIDENDRA was fortunate in coinciding with the publication of *Genera Orchidacearum*, the greatest effort ever made by the scientific community at producing a robust and natural account of the orchids at the generic level, incorporating molecular data in a truly phylogenetic classification, which eluded plant



FIGURE 9. Generic circumscriptions adopted by *Genera Orchidacearum* constitute the systematic framework of EPIDENDRA.


scientists for years (Pridgeon *et al.* 1999, 2001, 2003, 2005, 2009, in press) (Fig. 9). The editors invited several international specialists to contribute in their particular areas of expertise, to produce comprehensive treatments for each genus, with complete nomenclature, description, distribution, and summaries of our biological, ecologic, and economic knowledge. The first volume of the series saw the light in 1999, the second in 2001, and the third — including the second part of subfamily Orchidoideae and subfamily Vanilloideae — in 2003, just in time to be adopted as the general systematic framework of LBG's database. Since then, volumes 4 and 5 of *Genera Orchidacearum* (issued respectively in 2005 and at the end of 2009) presented the first two parts of the treatment of the largest subfamily of Orchidaceae, Epidendroideae, which also includes most Neotropical taxa. In particular, the last published volume includes treatments of 187 genera in tribe Cymbidieae. Many of the groups covered (like Maxillariinae and Oncidiinae) have been extensively reorganized in recent years, and the work updates accordingly generic circumscriptions and nomenclatural changes. In addition, the volume included an addendum with relevant nomenclatural changes in Laeliinae made since the publication of volume 4, in which that subtribe was covered. The

last volume of the series, volume 6, covering mostly Paleotropical genera, is expected to be published in 2014.

Even though orchid systematics will probably remain in a fluid state for years in the future as new data are incorporated, the framework of *Genera Orchidacearum* has proven to be consistently and broadly accepted by the scientific and horticultural communities, greatly reducing the effort by the staff working at EPIDENDRA to make nomenclatural assumptions on several alternative taxonomic circumscriptions.

#### Natural variation

Botanists working in tropical areas have an opportunity to improve our knowledge of orchid diversity and to provide a bridge between systematic research and the general public, incorporating in their floristic databases other data that are not accessible to their colleagues in the developed nations, such as visual databases of specimens, slides, drawings, etc. Systematically associated with preserved vouchers, and often with their respective pollinaria, the photographs of flowers included in EPIDENDRA are not representative of the species but instead a faithful depiction of individual characteristics and a tool to understand



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EPIDENDRA homepage

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***Kefersteinia parvilabris*** Schltr., *Repert. Sp. Nov. Regni Veg.* 19: 52. 1923.

TYPE: Costa Rica. San Jerónimo, Jan. 1922, C. Wercklé 116 [holotype, 8, destroyed; lectotype, selected by Pupulin (2001), AMES 31623, drawings of the holotype].

**HOMOTYPIC SYNONYMS**

*Chondrorhyncha parvilabris* (Schltr.) L.O. Williams, *Ceiba* 5: 195. 1956.

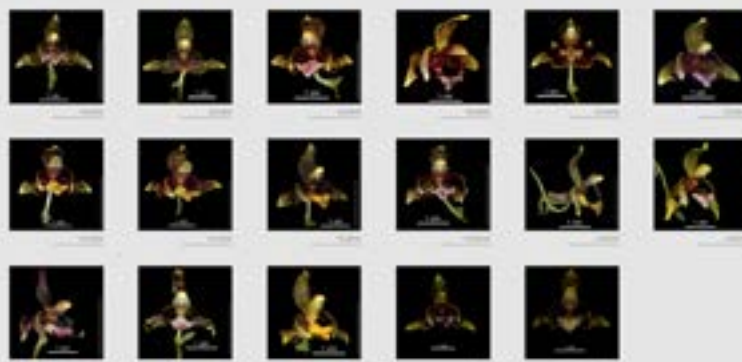
*Sephasia parvilabris* (Schltr.) Szlach., *J. Orchideenfreund* 10(4): 336. 2003.

**HETEROTYPIC SYNONYMS**


*Kefersteinia deflexipetala* Fowlie, *Orchid Digest* 30: 117. 1966.

TYPE: Costa Rica. Cartago: Cedral de Oro, 1400 m, C.H. Morich H64CR13 [holotype, LA (drawing of type); photo of paratype, AMES].


**PHOTOS AND SCANS**



**ILLUSTRATIONS**



**POLLINARIUM**



EPIDENDRA

FIGURE 10. A page from the actual on-line version of EPIDENDRA.

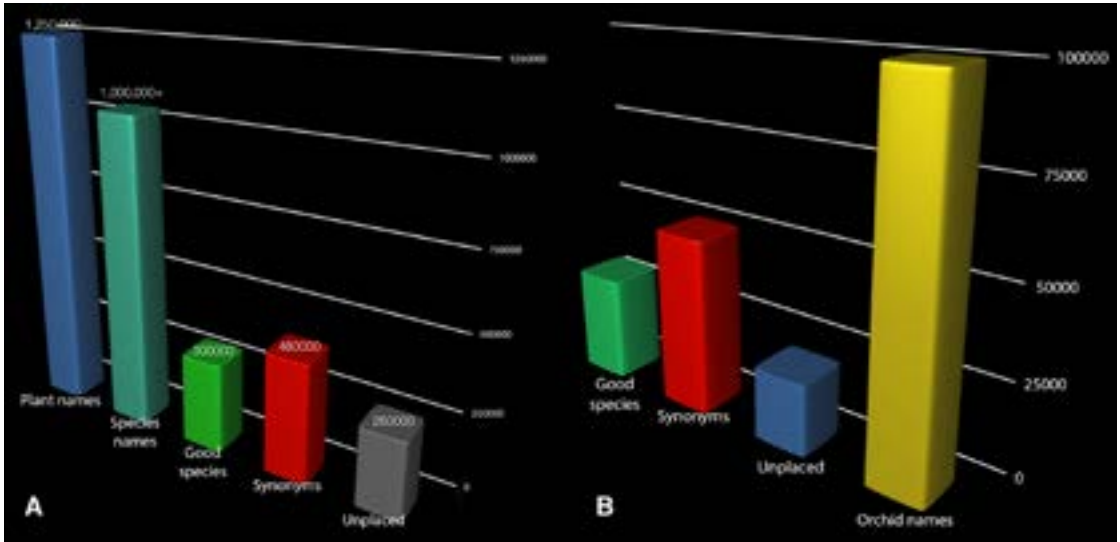


FIGURE 11. Published binomials in A) the plant kingdom, and B) Orchidaceae.

the natural variation of living organisms and improve our appreciation of specific circumscriptions (Pupulin 2009). Today, the database includes over 15,000 photographs and illustrations of orchid species. Looking at several files of EPIDENDRA such as those of *Guarianthe skinneri* (Bateman) Dressler & W. E. Higgins or *Kefersteina parvilabris* Schltr. (Fig. 10), one can easily understand why the taxonomy of tropical orchids, often based on the study of a single or a few specimens, is plagued by tens of thousands of synonymous names.

### A daunting task

Dealing with the nomenclature and taxonomy of probably the largest family of angiosperms represents a serious commitment. Current figures for the plant kingdom suggest a grand total of 1,250,000 names, of which about one million are species names. Of these, however, almost half are synonyms, and more than 250,000 are unplaced for lack of information or taxonomic verification (WCSP 2012; Fig. 11A). An estimation of the orchid names to be included in EPIDENDRA accounts for almost 100,000 binomials. Of these, probably less than 30,000 are good species, at least 55,000 are synonyms, and some 15,000 require further study to be placed correctly in any of the categories (Fig. 11B). Even though EPIDENDRA concentrates mostly on Neotropical taxa, the task remains daunting, as orchid species from tropical regions of the Americas represents half of all Orchidaceae. With some 8,000

binomials actually included, EPIDENDRA only covers today about one-tenth of the published orchid names (Fig. 12). However, coverage is not random: almost 90% of the orchid taxa from Costa Rica are included and approximately 40% of all Orchidaceae of Mesoamerica, including Mexico. Particularly well represented are also the orchids of Panama, which constitute a definite target for LBG researchers. Through the strong relationship developed recently with the Andean Orchids Research Center in Ecuador, emphasis has been put on Ecuadorian and Andean taxa, particularly in the most diverse group, Pleurothallidinae Lindl. (Fig. 13A).

Of the 16,000 users who visit EPIDENDRA every year, 85% come from Latin American countries. Of these, more than 40% are from Costa Rica and about 50% from Central America. Contacts from Colombia and Ecuador are most numerous from South America (5.5% and 3.5%, respectively), followed by visitors from Brazil (2.5%). The most frequent non-Latin American visitors are from the United States (7.5%), Germany (3.5%) and the Netherlands (3%).

### Where we are going from here

That EPIDENDRA could continue offering to the scientific and horticultural communities a real service is mostly dependent on the reliability of data presented in its pages. Until now, this has been achieved by focusing the effort of data taxonomic verification (species names, protologues, images



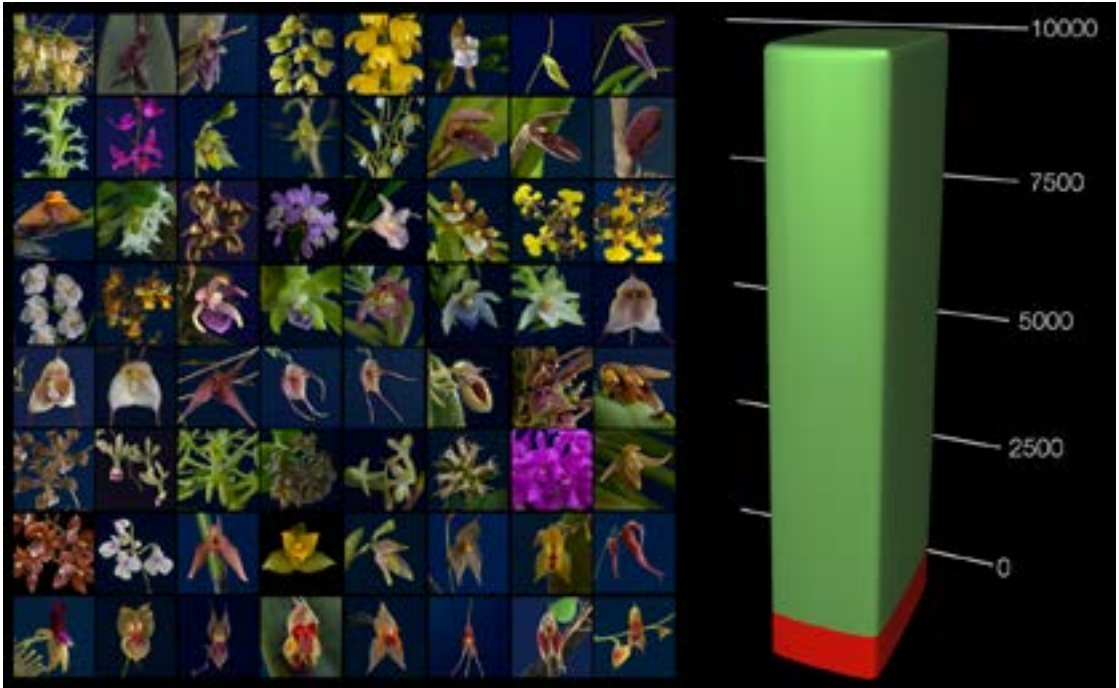


FIGURE 12. EPIDENDRA mostly includes records related to the orchid flora of the American tropics. With some 9,000 critically evaluated names, it covers today one-tenth of all Orchidaceae binomials.

of types, type illustrations, photographs and other illustrations) for those taxa that can be more easily studied at the center, both as living specimens and conserved vouchers, and on which the taxonomists of the LBG have more experience. Meanwhile, EPIDENDRA has been instrumental in the creation of a vast system of scientific agreements with other institutions and researchers. These include institutions that contributed digitized images of historically relevant materials and literature, as well as research centers and students that contribute their expertise in particular orchid groups or local floras (Fig. 14).

To expand geographic coverage of the database to embrace orchid floras of tropical regions of the Old World, and in particular the taxonomy of large groups as *Bulbophyllum* Thouars or *Dendrobium* Sw. or difficult species complexes as in the genera *Ophrys* L. and *Dactylorhiza* Neck. ex Nevski, the curators of EPIDENDRA need the scientific and technical support

from a large group of experienced researchers across the world. Such expertise, in fact, should not be limited to the taxonomy of the involved taxa but would require other skills to nourish the network with a rich and varied imaging support.

Given its actual coverage and the needs of local researchers, the first regions towards which EPIDENDRA should expand are the West Indies and the Amazonian regions and Brazil in South America (Fig. 13B). In the Old World tropical regions, the orchid flora of Africa will have priority in the next years, as it is likely that local botanists and naturalists will need original documentary sources to interpret and identify their plants correctly. Orchid-rich tropical regions of Southeast Asia will eventually represent the last step to complete the database (Fig. 13C). The complex orchid floras of the temperate regions are a minor priority for the global taxonomic orchid network, as scientists who deal with them also usually have unrestricted access to documentary sources.

Right: FIGURE 13. Covering areas of EPIDENDRA (2012) and directions for development. A – Most represented in the database are taxa from Central America and Andean South America. B – The Antilles, Amazonian South America and Brazil are the most immediate target of the network. C – Orchid species from the Paleotropics represent a further development.

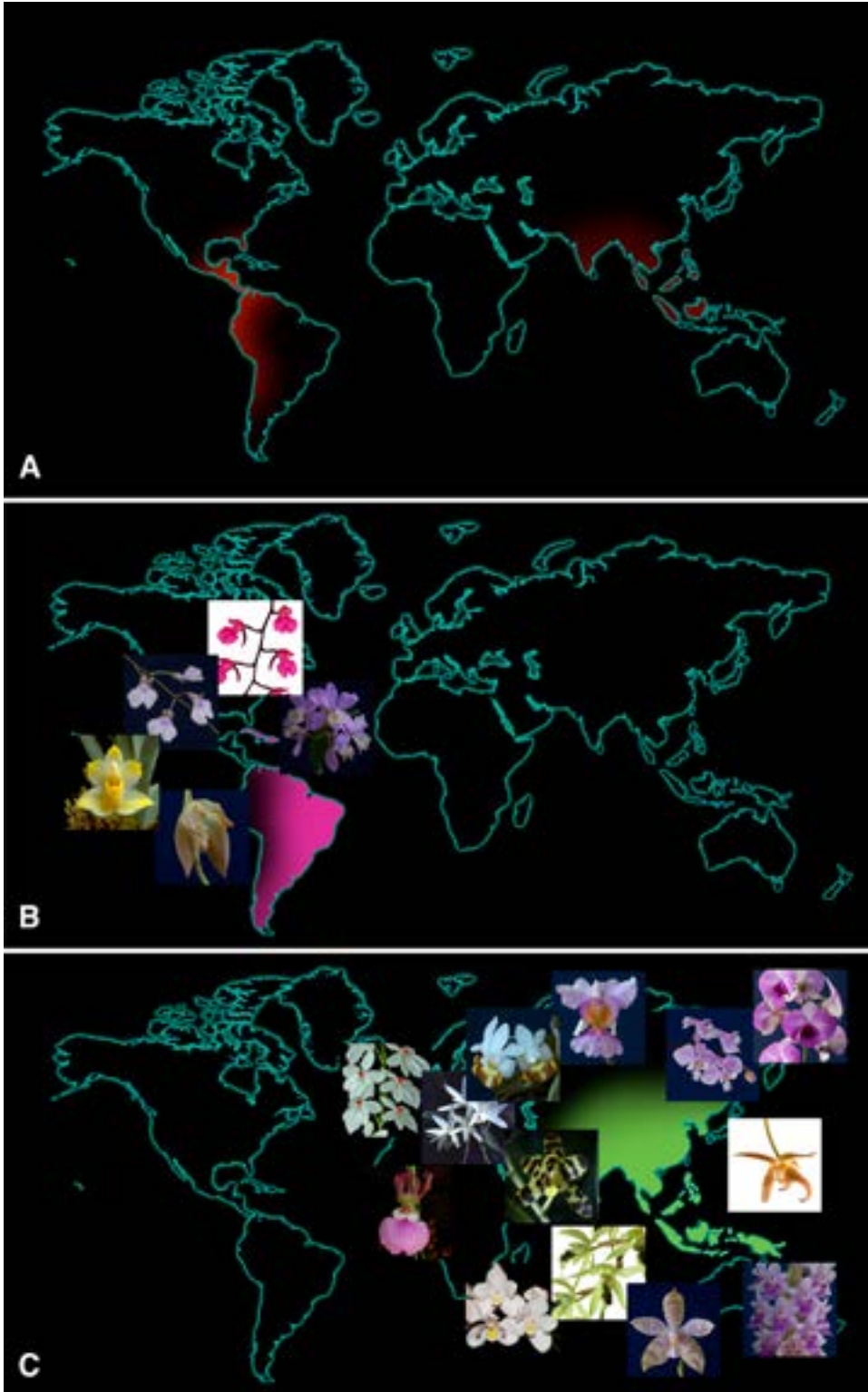




FIGURE 14. EPIDENDRA scientific network. Red dots indicate institutions that provide historical material and literature to the database. Green dots are centers providing expertise in particular groups. White dots are other institutions with which agreements for scientific cooperation are underway.

Progressive access to essential documents related to the orchid diversity of Costa Rica and other regions of Central America has enabled research staff at LBG to improve the quantity and quality of their taxonomic work significantly. The daily use of EPIDENDRA by a growing number of orchid researchers in the Latin America demonstrates that the documentary gap that slowed botanical activity in the tropics may be, and must be, left behind.

ACKNOWLEDGMENTS. I would thank all the people who participate to the conceptual discussion about the reasons and scopes of EPIDENDRA, and in particular Jorge Warner, Diego Bogarín, Daniel Jiménez, Hilda León-Páez, Carlos Ossenbach and Walter Schug. Gratitude is extended to my colleagues at LBG, Robert L. Dressler, Melania Fernández, Adam P. Karremans and Christina M. Smith, who daily improve the databases with their work. A special acknowledgment is due to the curators and staff of the herbaria and libraries that took part to the project in its ten years of activity: in particular to Gustavo A. Romero-González (AMES), Phillip J. Cribb (K), M. Velayos Rodríguez and M. R. Noya Santos (MA), Wesley E. Higgins and Bruce Holst (SEL), and Ernst Vitek (W). The project 814-BO-709 “EPIDENDRA: las bases de datos electrónicas de orquídeas del Jardín Botánico Lankester” is supported by the Vice-presidency of Research, UCR, which is warmly acknowledged here.

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## ORCHID CONSERVATION: THE NEXT TEN YEARS

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**ABSTRACT.** In 1996 the IUCN/SSC Orchid Specialist Group published a Status Survey and Conservation Action Plan that included a number of recommendations designed to address the problem of a projected imminent and rapid decline of species. Orchids remain subject to a multiplicity of threats in their natural habitats and, in addition to the usual suspects of habitat loss and land conversion, climate change has exerted a measurable effect on some orchid populations. Collection of orchids for traditional medicine is having a significant effect both within China and surrounding countries. Some progress has been made in achieving the targets set for Red Listing of endangered species in some countries, but other countries are lagging behind. In addition to successful initiatives in preserving natural habitats, a number of projects around the world have demonstrated that it is possible both to bring endangered species into cultivation and successfully reintroduce them into the wild. Orchid Seed Stores for Sustainable Use, with its goal of storing seeds representing a minimum of 1000 orchid species in institutes in at least 30 countries over the next five years, provides an example of what can be achieved by a small, dedicated group determined to share their technical expertise and develop a deeper understanding of the underlying science. There is, however, also an urgent need to promote awareness in the wider community about the importance of all sorts of biodiversity (not only orchids) through educational programs and involve people from as wide a range of backgrounds as possible..

**KEY WORDS:** seed storage, habitat, reintroduction, germination, collection

Environmental degradation attributable to human activity has been happening for centuries throughout the world. Islands have been particularly prone to such depredations. Pickering (2010) wrote that “A modern visitor to Mauritius, drawn by its perfect beaches or the wonders of its marine life, can have little idea of the paradise into which Van Neck’s men (who claimed the island for the Dutch in 1598) had stumbled. Mauritius was clothed in dense forest of ebony and bamboo and filled with bewildering wildlife. There were over twenty types of bird found nowhere else in the world, including, of course, the dodo.” Today a paltry 1% remains of the original forest.

In China the extinction of wildlife and wild places began much earlier. Slow at first, the degradation gradually accelerated and “an environmental richness counterbalanced by perpetual dangers from wildlife” was replaced by “a sedentized human dominance accompanied by a relative security from wild animals, but also ... *an impoverished life of the senses* (our emphasis)” (Elvin 2004). It is difficult to believe that in

times past there were elephants as far north as Beijing and rhinoceroses were almost as widespread.

Although today we tend to associate deforestation with human activities in the tropics, it is worth remembering that in England in 1712 John Morton summed up the prevailing attitudes when he proclaimed: “In a country full of civilised inhabitants timber could not be suffered to grow. It must give way to fields and pastures that are of more immediate use and concern to life.” When the Plymouth Brethren landed in North America they found “a hideous and desolate wilderness ... the whole country was full of woods and thickets;” and they set about civilizing it with chilling thoroughness. Within two centuries their descendents had obliterated seven-eighths of the continent’s natural woodland (and species such as the passenger pigeon) with an arrogance rivaling those in modern Amazonia (Mabey 1999).

Accounts of the enormous collections and imports of orchids in Victorian times make disturbing reading. Rittershausen (1993) quoted two authors

from the early days of the *Orchid Review*: “Few, if any, of the admirers of Orchids, ‘who sit at home at ease’, have any idea of the extent of the wholesale destruction of these plants which occurs in the process of their collection, particularly in Venezuela, and in the collection of cattleyas. Valleys are denuded of their primeval growths, for the forest giants, and the highest branches of some of these, is the home for some species of *Cattleya*, particularly the ever-varying forms of *Cattleya gaskelliana*. In order to secure the best plants these trees are felled, and from the harvest thus procured only the very finest plants are taken, the smaller ones and seedlings being left to perish with their natural support on the ground” (1897). Another author wrote, “Of *Cattleya labiata* Pernambuco exported about 15,000 plants of eight leaves and upward during the season ending with April, 1909” (1910).

The losses continue. Orchids today remain subject to a multiplicity of threats in their natural habitats and, in addition to the usual suspects of habitat loss, land conversion and illegal collecting, it seems probable that epiphytes will be exceptionally sensitive to climate change (Benzing 2004), which has already had a measurable effect on some orchid populations (Koopowitz & Hawkins 2012). Collection of orchids for traditional medicine is having a significant effect both within China and surrounding countries (Pant & Raskoti 2013). The U.K. has lost 97% of its flower-rich meadows and grasslands in the last 50 years (Walker *et al.* 2004). When describing habitats around Puerto Vallarta, Mexico, Warford (1989) reported that “A woman born in El Tuito, a small town some 27 miles (44 kilometers) south of Puerto Vallarta, tells of the time, scarcely 22 years ago, when Highway 200 was lined every mid-October with magenta clouds of *Schomburgkia galeottiana* plants in flower. Driving down the highway today, not a single specimen of that magnificent species can be seen.”

As recently as the 1970s, nursery benches in the U.K. were full of wild-collected species such as *Paphiopedilum fairrieanum* (Lindl.) Stein, which is currently reduced to one population. *Paphiopedilum vietnamense* O.Gruss & Perner was discovered in northern Vietnam in 1997. “All known populations in the wild have been depleted by illegal collection since its discovery” (Perner 2012).

### The fate of orchids

So what has gone wrong? Why are we still allowing this continuing loss of biodiversity in general, and of orchids in particular, to happen? Given the evidence it can be difficult to understand why so few people even notice. Still fewer appear to care. At least part of the answer may lie in what has been called the ‘Shifting Baseline Syndrome’, a phrase first coined by Daniel Pauly (Arbesman 2012) to explain the collapse of fish stocks off the shores of Newfoundland and Cape Cod. This indicates that each new generation accepts the state of the planet they see around them as being the norm and uses that baseline to evaluate changes in the environment taking place in its lifetime. It is only when you read the accounts of past authors such as Darwin and Wallace that the full scale of what we have already lost becomes all too apparent.

All that remains of the dodo in Oxford’s Natural History Museum are the head and the bones of a foot. One can only wonder if this is going to be the fate of many orchid species in the not-too-distant future. How many orchids are fated to be remembered only as dried pressed specimens in herbaria? Or are our orchids going to be like Brazil’s beautiful blue Spix’s macaw (*Cyanopsitta spixii* (Wagler)), which is now found only in captivity (Juniper & Yamashita 1990; Juniper 2002).

Environmentalists around the globe were delighted when Barack Obama vowed in his inaugural address to his second term of office to put climate change center stage in his next term as U.S. President, saying “We will respond to the threat of climate change, knowing that the failure to do so would betray our children and future generations.” On 23 August 2012, Robert Watson (past president of the Intergovernmental Panel on Climate Change – IPCC) appeared on television to suggest that we are extremely unlikely to meet the target of limiting global average temperatures to a 2°C increase; more likely it will be higher. On 16 September 2012, Arctic ice shrank to its lowest seasonal minimum since records began in 1979 (Pearce *et al.* 2012). We know that climate change is also affecting orchid populations. In his landmark 32-year study of a population of *Ophrys sphegodes* Mill., Hutchings (2010) found that, given some variation, peak flowering advanced by about half a day per year during the study period. In addition, fertilization rates of *O. sphegodes* are low in the U.K. and, although *O. sphegodes* is facultatively autogamous,

it also is cross-pollinated by a rare solitary bee. Unless the phenologies of *O. sphegodes* and the bee respond similarly to climate change, there is a risk that rates of pollination will be reduced in the future .

### Habitat conservation

Ideally the problem of conservation would be solved by preserving habitats such as El Pahuma in Ecuador (Seaton 2005) and La Planada Nature Reserve in Colombia (Orejuela Gärtner 2011), bearing in mind that habitats themselves may be subject to future change. It is unlikely, however, that it is going to be possible to conserve all habitats, and we should perhaps begin to examine our priorities and begin to identify Important Orchid Areas (IOAs) in a similar way to areas that have been identified as being Important Plant Areas (IPAs) (Anderson 2002; Foster *et al.* 2012).

Orchids are, of course, just one component of a complex physical and biological web of relationships that constitute a particular ecosystem. A species may be adapted for example to a particular temperature range and relative humidity. In order to germinate in their natural environment, seeds need to encounter a particular mycorrhizal fungus and may be reliant upon a different fungus at a subsequent life stage. They often need a specific pollinator, which in turn will be reliant on a particular set of plants to complete their life cycle. The relationships of orchid flowers with their insect pollinators is often intricate. As temperature and precipitation change, we do not know whether there will be synchrony of phenologies between orchids and their pollinators (Hutchings 2010) or what the potential consequences may be for their mutual survival. The majority of tropical orchids are epiphytes, growing in tree canopies. Recent findings on the adverse effects of climate change on populations of large and old trees indicate that loss of such trees could have a disastrous effect on certain epiphytic species (Laurance 2012). Whigham (pers. comm.) suggested that orchids are particularly sensitive to environmental change and could be used as indicators of change and act as bio-indicators/canaries in the coalmine.

### Reintroductions

There are, nevertheless, reasons to be optimistic. More people are participating in orchid reintroductions

around the world, including a number of the partners of Orchid Seed Stores for Sustainable Use (OSSSU). There are, for example, reintroduction programs for *Cattleya quadricolor* B.S.Williams in Colombia (Seaton & Orejuela Gärtner 2009), *Cyrtopodium punctatum* (L.) Lindl. in the Fakahatchee Strand in Florida, U.S.A. (Ferreira *et al.* 2012), *Cymbidium finlaysonianum* Lindl. and *Grammatophyllum speciosum* Blume in Singapore (Yam *et al.* 2010) and *Cypripedium macranthos* Sw. in China (Seaton 2010). The Royal Botanic Gardens, Kew, is participating in reintroduction projects for the critically endangered endemics *Epidendrum montserratense* Nir in Montserrat and *Angraecum longicalcar* (Bossler) Senghas in Madagascar. Montserrat's Soufrière Hills volcano, dormant for centuries, erupted in 1995 and is still active today. The eruption triggered pyroclastic flows, destroying large areas of forest. One area in the western part of the island where *E. montserratense* is commonly found still suffers from ash clouds, venting from the active volcano. Not only does the hot ash damage the host trees, but it also blocks rivers, causing flash floods, which destroy river valley vegetation. Another area of suitable habitat, in the Silver Hills in northern Montserrat, has largely escaped volcanic damage but has become even more important for agriculture and building land for the island's remaining human population. Two collections of seeds from *E. montserratense* are now safely stored in Kew's Millennium Seed Bank. Living plants, rescued from dead mango (*Mangifera indica* L.) trees, have been taken to the Montserrat National Trust's newly developed botanic garden where they are being cultivated for future display, and seedlings have been cultivated in Kew's Conservation Biotechnology section (CB).

The problem in Madagascar is of a different nature. Recent expeditions to investigate the status of *A. longicalcar* in its natural habitat found that just nine clumps remained: six on a ridge beyond a village in central Madagascar's Hauts-Plateaux (three producing fruiting spikes) and three between the village and a road. Those nearest the road were in a poor state, and one clump had suffered illegal collection; about ten large shoots had been cut off the rocks, leaving three small shoots. Another of the three clumps had been reduced in size considerably and was at risk eventually

of falling off the cliff top on which it was growing. Several plants that have been raised from seedlings at Parc Botanique et Zoologique de Tsimbazaza (PBZT) and transferred to a shade house in the village were subsequently planted out two years ago on a ridge of similar habitat to the ones on the ridge. These have established well, with many large, deep green leaves. The project is in the early stages and will run for several years. It is both an extension of previous work on methods for collecting highly germinable immature seed and part of a new collaborative project involving the use of ecological and mycorrhizal information to facilitate the reintroduction of threatened species. Seed baits and the collection of mature roots are being employed to identify and culture mycorrhizal fungi. Material (seed, seed pods, and roots) has been cultured, and seedlings are being raised in CB at Kew (<http://www.kew.org/about-kew/press-media/press-releases-kew/madagascar-orchid-conservation-illinois-college>). The involvement of the local community is key in maintaining and protecting the sites in the long term. After growing on in the shade house, the village children are reintroducing plants previously raised at PBZT. They each have individual plants that they care for and have been given project t-shirts. This local involvement has helped with engagement and is an important part of the species' management, which now includes husbandry of reintroduced plants and creation and maintenance of fire breaks around the sites.

### **Orchid Seed Stores for Sustainable Use (OSSSU)**

OSSSU, with its goal of storing seeds representing a minimum of 1000 orchid species in institutes in at least 30 countries by 2016, demonstrates what can be achieved by a small, dedicated group determined to create a global network of orchid seed banks, exchange technical expertise, and develop a deeper understanding of the underlying science. Initially focusing on biodiversity hotspots in Latin America and Southeast Asia, this Darwin Initiative (Defra, U.K.) project commenced in 2007, with regional workshops held in Chengdu, China, in October and Quito, Ecuador, in November of the same year. The network soon expanded to encompass other tropical regions and also countries with temperate climates. A four-day workshop for participating countries was held at Jardín Botánico Lankester, Costa Rica, in September

2010 to summarize the work carried out thus far and plan the next stage.

Good communication is key in setting up and maintaining such a broadly based coalition, and additional meetings take place whenever and wherever the opportunity arises. Thus colleagues from botanic gardens, research institutes, and universities from around China were able to meet at the National Orchid Conservation Center in Shenzhen in April 2012 to discuss the development of an in-country program that would support the conservation of their 1350 orchid species. Likewise, representatives from 11 participating countries held a workshop at the 8<sup>th</sup> International Orchid Workshop held at Orquideario Soroa, Cuba, in October 2011. From the original 15 countries in Latin America and Asia (plus the U.K.) the network has continued to expand, with 37 institutions in 25 countries participating to date.

Although a number of institutes in participating countries have the resources to collect material from the wild, for practical reasons OSSSU is primarily focused on living collections, and these play a key role in *ex situ* conservation efforts. However, it is vital that seed is collected from as broad a genetic base as possible, and selfing of plants is only used as a last resort when there is only one rare plant available, for example. Where possible, it would be desirable to establish collections containing significant numbers of plants of particular targeted species to reflect the genetic diversity within those chosen species.

It is important to recognize that conservation is not just a problem for scientists and, if we are to succeed in our mission to save orchids for future generations, we need to involve groups beyond the scientific community and promote a wider interest in conservation through Citizen Science projects. To this end OSSSU has obtained funding to host two workshops for holders of the National Collections of Orchids in the U.K. with the aim of encouraging them to pollinate their unique plants and collect seed for storage and research at Kew's Millennium Seed Bank. The National Plant Collection® scheme is hosted by Plant Heritage, the U.K.'s premier plant conservation charity. Individuals or organizations undertake to document, develop, and preserve a comprehensive collection of one group of plants in trust for the future. For example, the National Collection of *Maxillaria*

currently holds approximately 275 different species. The collection is underpinned by an impressive series of volumes of drawings and notes on each species. Each plant is photographed when in flower, and there is a comprehensive slide collection that is currently being digitized. A few plants are also represented as paintings. Kew has provided the herbarium sheets for pressed specimens, and there is a corresponding spirit collection of flowers. All of these are destined for Kew's Herbarium as an important resource for future investigators (Seaton 2011). Collections have the potential to act as important educational tools, increasing awareness of the enormous diversity of the orchid family among orchid growers and the general public.

A major opportunity to conserve orchids is evident in the storage of their seed. Many species produce enormous numbers of tiny seeds allowing the conservation of large numbers in comparatively small volumes. This can both ensure the retention of genetic diversity and potentially facilitate the long-term survival of these seeds under suitable conditions. Orchid seeds are considered to display an orthodox behaviour and extended longevity when subjected to drying (<5% moisture content) and freezing (-20° C) under the conditions commonly utilized in many seed banks. Evidence from the initial phase of the OSSSU, where participating laboratories used the same conditions to dry, store, and germinate seeds, showed that maintaining a high level of viability could vary among species stored under these conditions. A range of *Coeloglyne* species lost their viability, whereas *Dendrobium* species showed a mixed response when germinated on Knudson C (Knudson 1946) medium after 12 months of storage at -20° C (Table 1). Species of *Vandopsis*, *Phalaenopsis*, *Cymbidium*, and *Arundina* all retained maximum viability over some 18 months.

The suitability of this storage regime is part of ongoing research in a range of species to determine the effects of temperature and seed moisture content, both of which are recognized as major factors that affect the duration over which seeds can be successfully conserved. For example, in the temperate terrestrial *Gavilea littoralis* (Phil.) M.N.Correa, in which moisture content was reduced to c. 3% w/w through equilibration at 15% relative humidity, longevity was significantly promoted by storage in liquid nitrogen compared to that at 5° C (Table 2). However, such a temperature response can be modified by moisture content, as seen in the epiphytic species *Guarianthe aurantiaca* (Bateman ex Lindl.) Dressler & W.E.Higgins (Seaton & Hailes 1989), and in a range of Australian terrestrials (Hay *et al.* 2010). Specific sets of conditions can be identified that enhance the longevity of particular species, although the application of a more universal recommendation as a possible alternative to seed-banking conditions and applicability to a broader range of species await resolution. By working with a broad range of species, OSSSU partners are producing data that will increase our understanding of the basis of sustained longevity when orchid seed is stored at -20° C and, where possible, other temperatures.

Expression of seed viability can be measured in several ways. Partners of OSSSU have developed *in vitro* conditions for the successful asymbiotic germination of c. 240 species on a common medium (Knudson C). This is the first time that a large-scale comparison has been made using common procedures across a broad range of orchid species. Using a vital stain such as triphenyl tetrazolium chloride (TZ) establishes a base-line potential for germination and is critical in the recognition of sub-optimal medium. However, the technique can yield variable results with different types of seeds, and to improve the

TABLE 1. Viability and germination of temperate terrestrial *Gavilea littoralis* (Phil.) M.N.Correa seeds following one year of storage at different temperatures after equilibration of seeds at 15% relative humidity.

	Seed storage temperature (°C)				
	-196	-70	-20	5	20
Tetrazolium viability (%)	68.3±2.6				
Pre-storage germination	41.1±4.5				
Germination on ½B5 and activated charcoal	46.9±5.1	35.4±2.3	35.2±5.8	29.5±4.6	14.9±1.4

TABLE 2. Changes in the germination of *Coelogyne*, *Dendrobium*, and *Xylobium* species on Knudson C medium after 9 or 12 months of storage at -20°C.

Species	Storage duration (months)		
	Initial	9	12
<i>Coelogyne asperata</i> Lindl.	35.4±1.7		1.9±0.5
<i>Coelogyne foerstermannii</i> Rchb.f.	81.5±3.4	5.4±1.4	
<i>Coelogyne pandurata</i> Lindl.	99.5±0.3		6.8±2.0
<i>Coelogyne rumphii</i> Lindl.	64.7±3.6	1.1±0.5	
<i>Dendrobium macrophyllum</i> A.Rich.	69.6±3.7		52.8±0.6
<i>Dendrobium mirbelianum</i> Gaudich.	94.5±0.3 <sup>a</sup>		0
<i>Dendrobium stratiotes</i> Rchb.f.	95.6±0.6		0.6±0.3
<i>Xylobium undulatum</i> (Ruiz & Pav.) Rolfe	94.0±1.9		81.3±1.7

<sup>a</sup>Germination following one month of storage at -20°C

TABLE 3. Seed yield and size characteristics of two terrestrial species bearing small capsules.

	Capsule DWT (mg)	Total seeds DWT (mg)	Mean seed DWT (μg)	Total seeds/capsule
<i>Stenoglottis fimbriata</i> Lindl.	4.8±0.4	1.9±0.2	0.7±0.1	2721±507
<i>Ponerorchis graminifolia</i> Rchb.f.	5.3±0.9	2.2±0.6	1.2±0.4	1722±134

development of the red colouring associated with this stain a sucrose pre-conditioning stage has been used, initially with *Cattleya* species (Hosomi *et al.* 2011). The effect of storage on seed vitality can also be seen in more subtle responses; the rate of germination was both lowered and increased across a range of *Cattleya* species following three months of storage at -20° C (Hosomi *et al.* 2012).

Sharing expertise has enabled partners to conserve enormous quantities of germplasm, potentially making material available for re-introduction programs. Seed production can be vast. The enormous *G. speciosum* capsules, weighing over 4g (fresh weight), can contain as many as two million seeds, but even the smaller capsules of *Aerides odorata* Lour. can contain over 200,000 seeds. This level of fecundity can vary and is dependent upon seed and capsule size, whereas the much smaller capsules of terrestrial species *Stenoglottis fimbriata* Lindl. and *Ponerorchis graminifolia* Rchb.f. produce many fewer seeds (Table 3). Bearing in mind that seed weights of 0.3- 24.0 μg have been identified across the family (Arditti & Ghani 2000), this may indicate that seed yields are equally

variable. The phenology of seed set and capsule maturation times have also been recorded to provide data for identifying suitable harvest times. Importantly, the point of mass seed maturity achieved at the end of capsule maturation is associated with a reduction in moisture content, which has been shown to be critical to successful storage in *Phalaenopsis amabilis* Lindl. hybrids (Schwallier *et al.* 2011). Taken from data for Brazil, Indonesia, Thailand, and Singapore, these times have varied from >50 to >500 days and could provide base data for potential differences related to climate change.

### The next ten years

Although the problems faced by the natural world remain as serious as ever, we believe that it is a mistake to focus on these problems and not talk about the good things that are taking place. Quite simply the general public are turned off by all the doom and gloom. We need to share more positive stories to encourage people to engage with the problems. And people love orchids. Perhaps this is nowhere more evident than in Colombia, where the Ministry of Environment designated 2010



as the National Year of Orchids, mentioning in the resolution that during the last 100 years *Cattleya trianae* Linden & Rchb.f., Colombia's national flower, had lost 80% of its natural habitat. Happily, as with *A. longicalcar*, *C. trianae* is still cultivated by the local people in Colombia who assiduously protect their plants. On the other side of the Atlantic in South Africa, the Darling Wildflower Show has been held annually since 1917 and attracts thousands of visitors each year.

In 1996 the IUCN/SSC Orchid Specialist Group (OSG) published a Status Survey and Conservation Action Plan (Hagsater & Dumont 1996) that included a number of recommendations designed to address the problem of the projected imminent and rapid decline of species. Progress towards achieving some of these goals has perhaps been sporadic but nevertheless encouraging. In addition to OSSSU there are many groups active around the world. The OSG (<http://iucn.org.orchid>) itself continues to hold meetings in association with conferences at which members are attending, most recently in the U.K., Ecuador, the Czech Republic, Australia, and Singapore. The wide geographical spread of these meetings has allowed different groups of members to attend at least one meeting. Some progress has been made in achieving the targets set for Red Listing of endangered species in some countries, but other countries are lagging behind.

Deciding which species to preserve remains an *ad hoc* affair. According to the 'Noah Principle', all species are fundamentally equal, and everything should be saved regardless of its importance to humans; but perhaps we need to be more realistic and target particular species and habitats. We might also consider preserving the germplasm of some of the rarer varieties. They should be cloned before they die and are lost forever. Although orchids often are able to live for decades if not more than a century, some are short-lived in cultivation. A continuous production of high-quality specimens can be maintained using *in vitro* techniques with material conserved in a network of seed banks (Seaton 2007; Seaton & Pritchard 2008).

One current focus for developing OSSSU is in the biodiversity hotspots of southern China where, in addition to their susceptibility to climate change, many species of *Cypripedium* and *Paphiopedilum* (horticultural value) and *Dendrobium* and *Cymbidium* (horticultural and medicinal value) are under threat from

over-collection and habitat degradation from changes in land usage. Changes to vegetational zoning up mountain sides can also have dramatic effects upon the distribution of some species. Under rapidly changing pressures to adapt, certain species will inevitably die and may become extinct in the wild. By conserving the Chinese species as seed or as living collections and improving our understanding of the seed biology of this iconic family of plants worldwide, we have a greater opportunity to conserve many of these charismatic plants for future generations to use in horticulture and traditional medicine (Seaton *et al.* 2010).

There is no doubt that OSSSU has enabled more students to become engaged in orchid research activities, and it is no exaggeration to say that such projects can change people's lives. There is a need to find funding for more young people to attend and participate in conferences. We need to be engaging with a much wider audience outside the orchid community, explaining the importance of all sorts of biodiversity (not only orchids) through educational programs, involving people from as wide a range of backgrounds as possible, and sharing knowledge and delivering courses. The list of the 100 most threatened species as suggested by IUCN Species Specialist Groups (Baillie & Butcher 2012) named the orchids *Dendrophylax fawcettii* Rolfe and *Rhizanthella gardneri* R.S. Rogers as representatives of Orchidaceae.

We need to approach the task with imagination. The Orchid Conservation Alliance (OCA) continues to raise funds to buy land for conservation in South America. Monies raised by Orchid Conservation International (OCI) have been used to fund conservation projects and also enabled production of stunning posters by students on the Natural History Illustration degree course at Blackpool and the Fylde College in the U.K. The first poster, illustrated by Ian Cartwright, told the story of *Coryanthes kaiseriana* G. Gerlach and its pollination by euglossine bees. The second poster, by Ruth Grant, showed *Paphiopedilum rothschildianum* (Rchb.f.) Stein in its natural habitat in Borneo. Above all we need to develop inspirational educational programs (Light *et al.* 2003) that teach schoolchildren about the wonders of the natural world and the opportunities for exciting careers whether based in a laboratory environment or studying orchids in the field.

There is no shortage of research topics: pollination, inbreeding depression, and self-incompatibility, for example. Although not necessary in a horticultural context, the survival of reintroduced terrestrial orchids may be more successful if symbiotically raised material is used (Ramsay & Dixon 2003). There is an urgent need for more field studies, particularly in the tropics, to establish the baseline data for orchid populations that will allow us to monitor future changes. The orchid world would certainly benefit from better coordination of dissemination of information about who is doing what and where.

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## DIVERSIDAD DE ORQUÍDEAS DE LAS DIFERENTES FORMACIONES VEGETALES DE LOS ANDES PERUANOS

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**RESUMEN.** Debido a la presencia de la Cordillera de los Andes y la Corriente de Humboldt, el Perú posee una gran diversidad de ecosistemas. Mientras que las regiones bajas al oeste de los Andes peruanos está caracterizada por ser una franja desértica-semidesértica, con bosques secos, matorrales y principalmente formaciones vegetales estacionales, las regiones del lado este de los Andes se caracterizan por estar formados de extensos bosques húmedos de gran biodiversidad. A lo largo de la cordillera; la vegetación de la zonas altoandinas y de los valles interandinos del norte, centro y sur del Perú no son tan contrastantes, aunque si existen diferencias en la composición florística debido a la humedad; las regiones del norte son mas húmedas que las del centro y sur. Las orquídeas que se encuentran en los bosques montanos de la vertiente oriental de los Andes peruanos son principalmente epífitas y han sido objeto de numerosos estudios con el propósito de conocer su diversidad. En cambio el estudio de las orquídeas terrestres no han recibido la misma atención, a pesar de que éstas se encuentra en casi todos los ecosistemas del país, desde los desiertos, matorrales xerofíticos, turberas altoandinas e incluso en áreas donde se ha introducido especies exóticas, como los bosques de *Eucalyptus globulus*. En la presente contribución se describen las formaciones vegetales del desierto costero, valles interandinos y zonas altoandinas del Perú; así como las orquídeas que se han registrado en cada tipo de vegetación.

**ABSTRACT.** Due to the presence of the Andes Cordillera and the Humboldt Current, Peru has a great diversity of plant communities. For example, while the lowlands to the west of the Peruvian Andes are characterized mainly by being a semi-desert belt with dry forest, shrublands, and mostly seasonal vegetation formations, the eastern side of the Andes is covered by a vast wet forest of high biodiversity. In contrast to the lowlands on either side of the Andes, the Andean highlands throughout Peru are not as contrasting, although there are floristic differences because of diminishing humidity toward the south. Epiphytic orchids, which are distributed mainly in the montane forests of the eastern slopes of the Peruvian Andes, have been the goal of numerous botanical expeditions in order to document their diversity. By contrast, Peruvian terrestrial orchids have not received the same attention, although they are found throughout the country, from the deserts, xerophytic shrublands, wetlands at high elevations, even in areas where exotic species such as *Eucalyptus globulus* forest have been introduced. The present contribution describes the vegetation of the desert coast, inter-Andean valleys and highlands of Peru, as well as orchids that have been recorded in each vegetation type.

**PALABRAS CLAVE:** Orchidaceae, Perú, desierto costero, zonas altoandinas, valles interandinos

La Cordillera de los Andes y la Corriente de Humboldt hacen que el Perú albergue una gran diversidad biológica. La primera crea una variedad de zonas ecológicas entre sus estribaciones y la segunda modifica notablemente las condiciones térmicas y precipitaciones pluviales al lado oeste de los Andes, ocasionando la aridez de la costa peruana (Weberbauer 1945; Dillon *et al.* 2003).

Una característica de los Andes peruanos es la notoria diferencia que existe en la vegetación a ambos

lados de la cordillera. Mientras que las formaciones herbáceas estacionales, matorrales y bosques xerofíticos se desarrollan en la vertiente occidental, los bosques húmedos tropicales caracterizan la vertiente oriental.

La mayor diversidad de orquídeas se encuentran en los bosques de las vertientes orientales; éstas son principalmente epífitas (muchas con flores llamativas) y han sido por décadas el interés y objeto de investigación de muchos botánicos y aficionados

al cultivo de las orquídeas. Por tal razón existe más información de las orquídeas de esta región de los Andes peruanos, que de las vertientes occidentales, zonas altoandinas y valles interandinos. Con el propósito de incrementar el conocimiento de la diversidad, distribución y estado de conservación de las orquídeas terrestres de la costa y territorio Andino (sierra) del Perú; en los últimos años se ha ido procesando información a partir de trabajos de campo, revisión de herbarios (USM, MOL, HAO, HUT, CPUN y PRG) y datos proporcionados por diferentes botánicos peruanos. Así como consultas a las bases de datos online de F y MO en busca de registros adicionales. Producto de los datos procesados, a continuación se describe las diferentes formaciones vegetales de la costa, zonas altoandinas y valles interandinos; así como las orquídeas que se han reportado en cada una de ellas.

#### Vegetación de la costa

La costa peruana es una angosta faja desértica-semidesértica que se extiende desde 3°23' hasta los 18° latitud sur y ocupa aproximadamente el 10% del territorio peruano. La altura sobre el nivel del mar es variable, pero no sobrepasa los 1000 m de altitud (Ferreya 1983). La condición desértica-semidesértica en la costa está en íntima relación con la influencia de la Corriente fría de Humboldt y el efecto de sombra de la Cordillera de los Andes (Dillon *et al.* 2003).

La vegetación semidesértica de la zona costera comprendida entre los 4° y 8° S está influenciada por la ocurrencia de lluvias durante los meses de diciembre a marzo (verano); las cuales pueden ser muy abundantes durante la ocurrencia de los eventos El Niño y causar grandes inundaciones. En esta zona se observa un marcado estrato arbóreo y arbustivo xerofítico que puede o no perder las hojas durante la estación seca y un estrato herbáceo efímero, que solo aparece durante la época de lluvias. De acuerdo a la literatura y material de herbario que se ha revisado, en éstos bosques estacionalmente secos, hasta la fecha no se ha registrado especies de orquídeas. Esto quizá se debe a que las investigaciones en estas formaciones vegetales se han enfocado al estudio de las especies leñosas (Linares-Palomino 2004; Aguirre *et al.* 2006; La Torre-Cuadros & Linares-Palomino 2008).

A partir del grado 8° S, la costa peruana es una región

hiperárida más o menos continua que ocasionalmente es interrumpida por los ríos que descienden de la Cordillera de los Andes. En esta zona de los desiertos, a pesar de que la humedad atmosférica es alta todo el año, las precipitaciones son escasas y solo se presentan a manera de una lluvia fina interrumpida; por lo que la vegetación que se desarrolla es fundamentalmente de tipo herbácea. Las formaciones vegetales donde se han registrado orquídeas son:

*Formaciones de Lomas* — Son comunidades de plantas efímeras que se desarrollan en ciertas colinas cercanas al mar y que rara vez sobrepasan los 1000 m. de altitud. La vegetación se desarrolla entre los meses junio y octubre (invierno y parte de la primavera) debido a la condensación del manto de neblina que cubre el litoral peruano por efecto de la corriente de Humboldt (Ferreya 1983; Dillon *et al.* 2003). Aunque por lo general en el Perú las lomas se encuentran distribuidas discontinuamente entre los 8° y 18° S (Ferreya 1983, 1993), éste tipo de formación vegetal también se ha registrado más al norte, a los 6°52' S en el Cerro Reque (Weberbauer 1945; Dillon *et al.* 2003).

La vegetación de lomas está compuesta principalmente por especies herbáceas anuales y perennes (geófitas); aunque existen localidades con especies leñosas. La composición florística varía de una localidad a otra, así como su grado de desarrollo. En las lomas del norte llegan a su óptimo desarrollo en julio, mientras que las del sur en octubre-noviembre; dependiendo esto de la cantidad de precipitaciones invernales, la cual varía de un año a otro.

Con respecto a las orquídeas que se han registrado para las formaciones de lomas (Tabla 1), *Chloraea pavonii* es la más conocida y vistosa; ésta orquídea era frecuente en las lomas cercanas a la ciudad de Lima (capital del Perú), por lo que no pasaba desapercibida y fue colectada por los principales botánicos y naturalistas que han estudiado la flora peruana como H. Ruiz & J. Pavón, A. Raimondi, A. Weberbauer y R. Ferreya (Ruiz 1952; Pupulin 2012). En la actualidad esta orquídea parece haber desaparecido de las formaciones de lomas cercanas a la ciudad de Lima debido a la expansión urbana, siendo el último espécimen que se ha examinado en los herbarios el de Ferreya 8748 (USM) de 1952 en Lomas de Iguanil.

TABLA 1. Localidades de lomas donde se han registrado especies de orquídeas. Las localidades han sido ordenadas de norte a sur.

Localidad	Especies	Voucher / Referencia
<b>Departamento de Lambayeque</b>		
Cerro Reque	<i>Pterichis</i> sp.	Laos 2051 (USM) Laos 4574 (PRG) Laos 4603 (PRG) Llatas 342 (MO)
<b>Departamento de La Libertad</b>		
Cerro Campana	<i>Pelexia matucanensis</i> (Kraenzl.) Schltr.	Angulo 765 (HUT) López 710 (HUT, F)
Cerro Cabras	<i>Malaxis andicola</i> (Ridl.) Kuntze	Angulo s.n. (HUT)
	<i>Pelexia matucanensis</i> (Kraenzl.) Schltr.	Angulo 1309 (HUT)
<b>Departamento de Ancash</b>		
Lomas de Casma	<i>Pelexia matucanensis</i> (Kraenzl.) Schltr.	Ferreyra 8049 (USM, MOL, F)
Lomas de Mongon	<i>Pelexia matucanensis</i> (Kraenzl.) Schltr.	Leiva et al. 2008
<b>Departamento de Lima</b>		
Lomas de Lachay	<i>Aa weddelliana</i> (Rchb.f.) Schltr.	Cano 7101 (USM) WENT s.n. (MO)
Lomas de Iguanil (Chancay)	<i>Chloraea pavonii</i> Lindl.	Ferreyra 8748 (USM, F)
Lima <sup>a</sup>	<i>Chloraea pavonii</i> Lindl.	Raimondi 471 (W) Ruiz s.n. (G)
Amancaes	<i>Pelexia matucanensis</i> (Kraenzl.) Schltr.	Esposito s.n. (MOL)
	<i>Chloraea pavonii</i> Lindl.	Esposito s.n. (MOL) Weberbauer 746 (F foto)
San Jeronimo	<i>Chloraea pavonii</i> Lindl.	Maisch s.n. (USM)
Cerro Agustino	<i>Pelexia matucanensis</i> (Kraenzl.) Schltr.	Soukup 2045 (USM, F) Weberbauer 5696 (F)
Lomas de Atocongo	<i>Pelexia matucanensis</i> (Kraenzl.) Schltr.	Ferreyra 9514 (USM, F)
<b>Departamento de Ica</b>		
Lomas de Amara	<i>Aa weddelliana</i> (Rchb.f.) Schltr.	Orellana 353 (MOL)
Al sur de Nasca <sup>a</sup>	<i>Aa weddelliana</i> (Rchb.f.) Schltr.	Rahn 198 (USM)
<b>Departamento de Arequipa</b>		
Lomas de Los Cerrillos	<i>Aa weddelliana</i> (Rchb.f.) Schltr.	Ferreyra 13455 (USM)
Atiquipa	<i>Aa weddelliana</i> (Rchb.f.) Schltr.	Delgado 4021 Ferreyra 14034 (USM)

<sup>a</sup> El colector no indicó la localidad exacta.

Las demás orquídeas que se han reportado para lomas poseen flores menos conspicuas y por ende poco conocidas, estas son: *Aa weddelliana* (Fig. 1A), *Malaxis andicola* y *Pelexia matucanensis* (Schweinfurth 1958; Leiva et al. 2008; Trujillo & Delgado 2011).

La revisión de las colecciones de los herbarios USM y PRG evidencia la presencia de poblaciones de *Pterichis* sp. creciendo de 400 a 585 m de altitud entre *Tillandsia* sp. en las lomas del Cerro Reque (Departamento de Lambayeque). Este registro, no

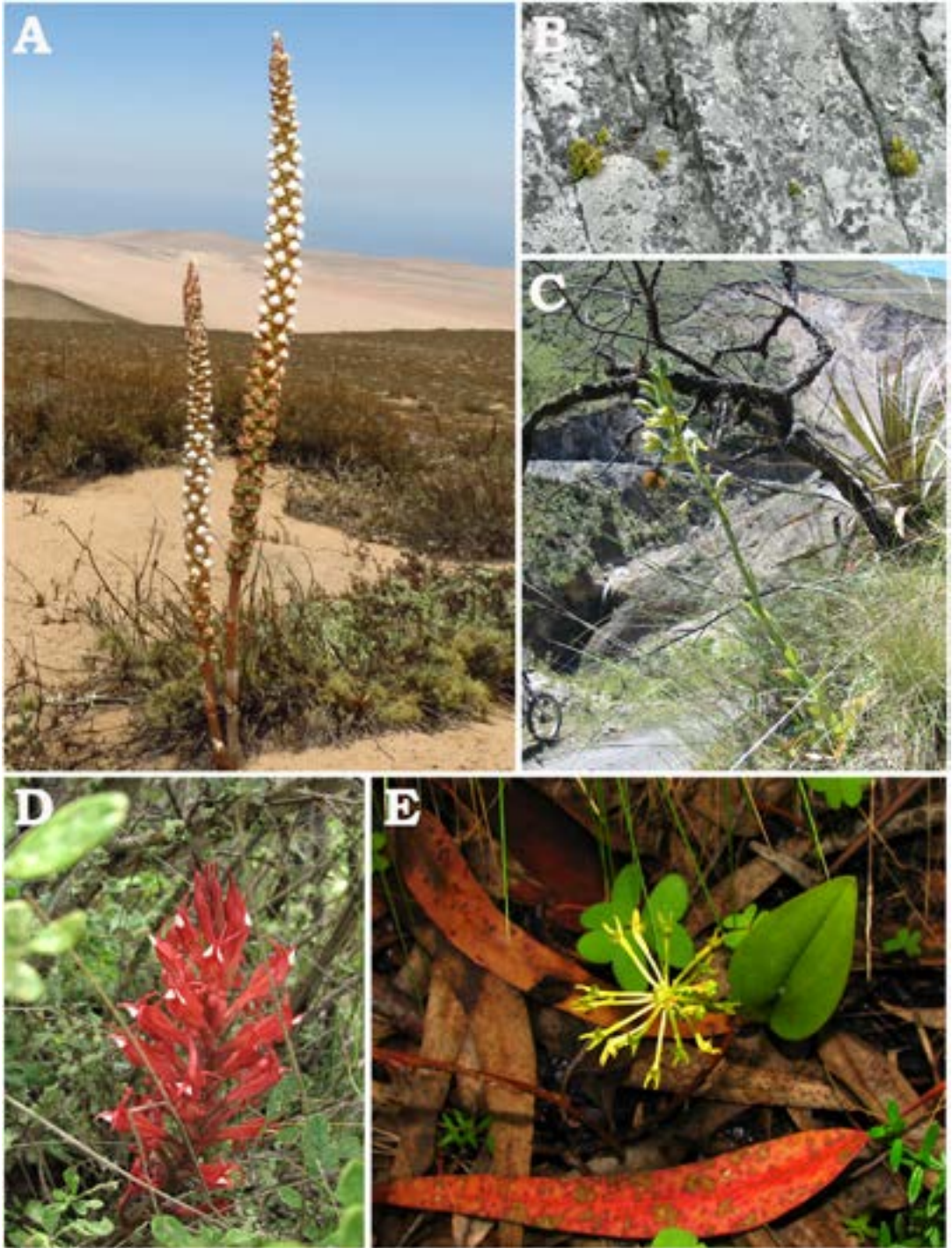


FIGURA 1. Fotografías de: A. *Aa weddelliana* en formación de lomas. B. Plantas de *Stelis* sp. creciendo sobre roca a ~ 4000 m en la sierra de La Liberad. C. *Chloraea septentrionalis* creciendo al borde del camino. D. *Porphyrostachys pilifera* creciendo bajo arbustos. E. *Malaxis andicola* creciendo en el sotobosque de una plantación de *Eucalyptus globulus*. Fotos: D. Trujillo.



sólo representa una nueva especie de orquídea para las formaciones de lomas del Perú, sino también permite ampliar el rango de distribución altitudinal del género *Pterichis*, que se pensaba que sólo se encontraban entre los 2800 a 4300 m de altitud (Cribb 2003).

*Tillandsiales* — Son comunidades vegetales muy especiales, constituidas por una o varias especies del género *Tillandsia* (Bromeliaceae) que habitan sobre las arenas de los desiertos entre 8°-18° S. Estas plantas tienen la particularidad de absorber el agua no por las raíces, que están atrofiadas, sino por las hojas a través de unos pelos escamosos; por lo que enteramente dependen de la humedad del ambiente (Ferreira 1983; Hesse 2012). Los *Tillandsiales* se encuentran en las colinas de suave pendiente, adyacentes al territorio de las lomas costeras a manera de una angosta faja gris discontinua, comúnmente ocupa áreas muy reducidas, aunque hay algunas que son de gran extensión. En ésta formación vegetal, la única orquídea que se ha registrado es una *Aa* sp. en los *Tillandsiales* de Marcona, Departamento de Ica (Vector-Peru 2009; C. Vargas, comm. pers.); es muy probable que la identidad de ésta especie sea *A. weddelliana*, que es la especie que se ha registrado en las formaciones de lomas del mismo departamento.

#### **Vegetación del territorio andino**

El territorio andino o sierra se extiende desde los 800 o 1000 m de altitud hasta el límite inferior de las nieves perpetuas (~5000 m). La diversidad florística de los Andes peruanos es el resultado de su compleja orografía y de la variedad de climas que existe a lo largo de la cordillera (Weberbauer 1945). Mientras que la abundancia de precipitaciones caracterizan a las vertientes orientales y se presentan prácticamente durante todo el año; en las vertientes occidentales (parte superior de ellas), las zonas altoandinas y los valles interandinos el régimen de precipitaciones es básicamente estacional (por lo general entre octubre y marzo). Por consiguiente, la vegetación que caracteriza a estas regiones es la de tipo xerofita o sub xerofita.

*Formaciones vegetales de la zona altoandina* — El límite inferior de la zona altoandina se encuentra entre los 3800-4000 m. de altitud en el Centro y Sur de los Andes peruanos y de 3000 m en el Norte. Esta zona es pobre en plantas leñosas y estas se

limitan (con excepción de unos cuantos arbustos) a determinados sitios, principalmente en superficies rocosas y pedregales. De acuerdo con el predominio de determinadas forma de vida y de crecimiento y a los tipos de suelo, se observan las siguientes formaciones vegetales (las orquídeas que se indican a continuación han sido registrados entre los 6° 30' y 9° 30' S en la Cordillera Occidental de los Andes):

*Pajonal* — Ésta formación vegetal es la más extensa de la zona altoandina. Está compuesta por asociaciones de gramíneas que crecen en manojos o macollos de hojas duras y punzantes que corresponde a los géneros: *Festuca*, *Stipa*, *Calamagrostis* y *Poa*. Las orquídeas que se han registrado en los pajonales son: *Aa aurantiaca*, *Aa erosa*, *Aa* sp., *Myrosmodes nubigenum*, *Myrosmodes paludosa*, *Myrosmodes* sp., *Pterichis triloba*, y *Pterichis* sp.

*Bofedal* — Esta comunidad vegetal ocupa los lugares semipantanosos o pantanosos de la zona altoandina. En algunos bofedales la especie dominante es *Distichia muscoides* Nees y Meyen (Juncaceae), que crece a manera de cojines convexos; varias hierbas pequeñas mas no arbustos pueden acompañar a las *Distichia*. En ésta formación vegetal, sólo especies del género *Myrosmodes* han sido registrados; por ejemplo: *Myrosmodes nubigenum*, *M. paludosa* y *M. pumilio*.

*Matorral* — Son formaciones abiertas de arbustos con escasos arboles, helechos y hierbas anuales y geófitos. Las orquídeas registradas en esta formación son: *Aa* sp., *Atensteynia fimbriata*, *Chloraea septentrionalis*, *Cranichis* sp., *Cyclopogon elatus*, *Cyrtochilum aureum*, *Elleanthus amethystinus*, *Elleanthus conifer*, *Epidendrum inamoenum*, *Epidendrum stictoglossum*, *Gomphichis macbridei*, *Gomphichis valida*, *Stenoptera montana*, *Stenoptera* aff. *peruviana*, *Masdevallia amabilis*, *Pleurothallis spiralis*, *Pleurothallis virgata*, *Pterichis triloba*, *Pterichis* sp., *Sudamerlycaste* sp., *Sauroglossum schweinfurthianum* y *Stelis* sp.

En matorrales que se encuentran cerca a pequeños ríos y quebradas a altitudes superiores a los 3500 m.; se ha registrado *Liparis elegantula* y *Malaxis andicola* creciendo bajo la sombra de algunos árboles y arbustos.

*Vegetación de los pedregales y roquedales* — Es la vegetación que se desarrolla en los acantilados,

aflorescencias rocosas y pedregales; está compuesta por líquenes, musgos, helechos, hierbas y algunos arbustos. Las orquídeas que habitan en estas formaciones crecen en la tierra que se acumula entre las piedras y en las fisuras de las rocas, y por lo general están protegidas por otras plantas. Las orquídeas registradas para esta formación vegetal son: *Aa* sp. *Chloraea septentrionalis*, *Cyrtorchilum aureum*, *Gomphichis valida*, *Elleanthus amethystinus*, *Epidendrum stictoglossum*, *Masdevallia amabilis*, *Masdevallia semiteres*, *Platystele rauhii*, *Pleurothallis virgata*, *Pterichis triloba*, *Stenoptera montana*, *Stenoptera* aff. *peruviana*, *Stelis* spp., *Sudamerlycaste* sp. y *Trichoceros tupaipi*.

También se han registrado orquídeas creciendo directamente sobre la roca (Fig. 1B), sin la protección de otras plantas, sólo líquenes y hay veces musgos están cubriendo sus raíces. Estas especies son: *Masdevallia semiteres*, *Pleurothallis virgata*, *Stelis* spp. y *Trichoceros tupaipi*.

**Bosques** -- De este tipo formación vegetal, la más importante es el bosque de *Polylepis* spp. Estas especies se desarrollan en ciertos valles altoandinos o laderas con suelos rocosos entre los 1800 y 5100 m (Mendoza & Cano 2011). En los lugares donde los individuos de *Polylepis* no son muy densos se han registrado las siguientes orquídeas: *Epidendrum stictoglossum*, *Gomphichis valida*, *Malaxis andicola*, *Masdevallia amabilis* y *Pleurothallis spiralis*.

Por encima de los 3700 m de altitud, en las ramas de *Polylepis weberbauer* Pilg. y *Buddleja incana* Ruiz & Pav. se ha registrado *Epidendrum excelsum*; esta especie es la única orquídea epífita altoandina registrada hasta el momento.

En algunos paisajes antropizados, es decir lugares donde la vegetación ha sido alterada por la actividad humana; también se han registrado orquídeas (Tabla 2). Las zonas de los paisajes antropizados donde se han colectado orquídeas son: los bordes y cercos de los campos de cultivo, bordes de caminos y muros de las construcciones precolombinas abandonadas. Entre las orquídeas registradas en estas zonas tenemos: *Altensteinia fimbriata*, *Chloraea septentrionalis* (Fig. 1C), *Cyclopogon elatus*, *Cyrtorchilum aureum*, *Stenoptera* aff. *peruviana*, *Masdevallia semiteres*, *Pleurothallis virgata* y *Stelis* sp. (el total de especies registradas se muestra en la Tabla 2).

**Formaciones vegetales de los valles interandinos** — Son los valles que se encuentran entre las estribaciones andinas entre los 900 y 3900 m. de altitud; por donde corren los ríos que se dirigen hacia la cuenca del Amazonas. La cantidad de agua que reciben estos valles es muy variada; algunos gozan de humedad durante todo el año, mientras que otros soportan moderados periodos de sequía. A esta última, corresponde la mayoría de los valles interandinos del Perú; por lo que el tipo de vegetación predominante es el matorral xerofítico.

**Matorral xerofítico** — Entre las especies leñosas que componen esta formación vegetal se encuentran las pertenecientes a los géneros *Acacia*, *Cercidium*, *Tecoma*, *Jatropha*, *Monnina*, *Rubus* y *Croton*, y entre las herbáceas varias Cactáceas y Bromeliáceas.

Como ejemplo de las orquídeas de los matorrales xerofíticos de los valles interandinos citare a las especies registradas en el valle del Huallaga entre los 2000 y 3150 m de altitud; estas son: *Aa* sp., *Altensteinia fimbriata*, *Cranichis ciliata*, *Epidendrum secundum*, *Malaxis andicola*, *Porphyrostachys pilifera* (Fig. 1D), *Pelexia* aff. *laxa*, *Ponthieva* aff. *pseudoracemosa*, *Sauroglossum schweinfurthianum* y *Trichoceros* sp.

Muchos de los matorrales de los valles interandinos han sido impactados por la actividad del ser humano, se estima que gran parte de estas formaciones surgieron como resultado de la alteración de los bosques a causa de la agricultura, el pastoreo y la extracción de madera (Sarmiento 2002). La forestación o reforestación de los valles interandinos con especies exóticas; como *Eucalyptus globulus* Labill., provoca cambios en la composición de la flora nativa. Sin embargo, el grado del impacto varía de acuerdo a la zona donde ocurren estos bosques y a las dimensiones de los mismos (FAO 2002). En el sotobosques de plantaciones de *E. globulus*, en cuyas cercanías todavía existían matorrales con especies nativas, se han registrado las siguientes orquídeas: *Cranichis ciliata*, *Malaxis andicola* (Fig. 1E), *Pelexia* aff. *laxa*, *Ponthieva* aff. *pseudoracemosa*, *Sauroglossum schweinfurthianum* y *Trichoceros* sp.

Todavía existen muchas localidades de los Andes peruanos donde no se han realizado estudios florísticos; y si estos se han realizado, no han reportado especies de Orchidaceae. Posiblemente, esto se debe a que muchas veces la época cuando se hicieron los trabajos

TABLA 2. Especies de orquídeas registradas para las diferentes formaciones vegetales de la zona altoandina y valle interandino. A.Alt = Área Alterada, Bof = Bofedal, B.Euc = Bosque de *Eucalyptus*, B.Poly = Bosque de *Polylepis*, Mat = Matorral, MatXero = Matorral xerofítico, Paj = Pajonal, P&R = Pedregal y Roquedal.

Especies	Zona altoandina						Valle interandino	
	Paj	Bof	Mat	P&R	B.Poly	A.Alt	MatXero	B.Euc
TRIBU CRANICHIDEAE								
<b>Subtribu Cranichidinae</b>								
<i>Cranichis ciliata</i> Kunth							x	x
<i>Cranichis</i> sp.			x			x		
<i>Ponthieva</i> aff. <i>pseudoracemosa</i> Garay							x	x
<i>Pterichis triloba</i> (Lindl.) Schltr.	x		x	x				
<i>Pterichis</i> sp.	x		x					
<b>Subtribu Prescottinae</b>								
<i>Aa aurantiaca</i> D.Trujillo	x							
<i>Aa erosa</i> (Rchb.f.) Schltr.	x							
<i>Aa</i> spp.	x		x	x		x	x	
<i>Altensteinia fimbriata</i> Kunth			x			x	x	
<i>Gomphichis macbridei</i> C. Schweinf.			x					
<i>Gomphichis valida</i> Rchb.f.			x	x	x	x		
<i>Myrosmodes nubigenum</i> Rchb.f.	x	x						
<i>Myrosmodes paludosa</i> (Rchb.f.) P.Ortiz	x	x						
<i>Myrosmodes pumilio</i> (Schltr.) C.A.Vargas		x						
<i>Myrosmodes</i> sp.	x	x						
<i>Porphyrostachys pilifera</i> (Kunth) Rchb.f.							x	
<i>Stenoptera</i> aff. <i>peruviana</i> C. Presl			x	x		x		
<i>Stenoptera montana</i> C. Schweinf.			x	x		x		
<b>Subtribu Spiranthiniae</b>								
<i>Cyclopogon elatus</i> (Sw.) Schltr.			x			x		
<i>Pelexia</i> aff. <i>laxa</i> (Poepp. & Endl.) Lindl.							x	x
<i>Sauroglossum schweinfurthianum</i> Garay			x			x	x	x
TRIBU MALAXIDEAE								
<i>Liparis elegantula</i> Kraenzl.			x					
<i>Malaxis andicola</i> (Ridl.) Kuntze			x		x		x	x
TRIBU CHLORAEAE								
<i>Chloraea septentrionalis</i> M.N. Correa			x	x		x		
TRIBU SOBRALIEAE								
<i>Elleanthus amethystinus</i> (Rchb.f. & Warsz.) Rchb.f.			x	x		x		
<i>Elleanthus conifer</i> (Rchb.f. & Warsz.) Rchb.f.			x					

TABLA 2. (continúa).

Especies	Zona altoandina						Valle interandino	
	Paj	Bof	Mat	P&R	B.Poly	A.Alt	MatXero	B.Euc
TRIBU EPIDENDREAE								
<b>Subtribu Laeliinae</b>								
<i>Epidendrum excelsum</i> C.Schweinf.					x	x		
<i>Epidendrum inamoenum</i> Kraenzl.			x					
<i>Epidendrum stictoglossum</i> Hágsater & D.Trujillo			x	x	x	x		
<i>Epidendrum secundum</i> Jacq.							x	
<b>Subtribu Pleurothallidinae</b>								
<i>Masdevallia amabilis</i> Rchb.f. & Warsz.			x	x	x			
<i>Masdevallia semiteres</i> Luer & R.Escobar				x		x		
<i>Platystele rauhii</i> Luer				x		x		
<i>Pleurothallis spiralis</i> (Ruiz & Pav.) Lindl.			x		x			
<i>Pleurothallis virgata</i> Luer			x	x		x		
<i>Stelis</i> spp.			x	x		x		
TRIBU CYMBIDIEAE								
<b>Subtribu Maxillariinae</b>								
<i>Sudamerlycaste</i> sp.			x	x		x		
<b>Subtribu Oncidiinae</b>								
<i>Cyrtorchilum aureum</i> (Lindl.) Senghas			x	x		x		
<i>Trichoceros tupaipi</i> Rchb.f.				x		x		
<i>Trichoceros</i> sp.							x	x

de campo no coincidieron con la época de floración de las orquídeas, o porque no se exploraron bien las formaciones vegetales dónde estas especies habitan. Algunas veces aunque se han observado especies de orquídeas en ciertas localidades, el material colectado no fue revisado por los especialistas de la familia y no se determinaron las especies; o simplemente no se prepararon especímenes de herbario. Todo esto ha dado como resultado, que exista poca información sobre la riqueza de especies y se subestime la diversidad de orquídeas del territorio andino y costero.

El primer paso para conservar las orquídeas de los Andes peruanos es conocer su diversidad y distribución; para ello es necesario muchos estudios de investigación básica que incluyan exploraciones botánicas en todas las formaciones vegetales; incluso en aquellas que han sido alteradas por el hombre.

AGRADECIMIENTOS. A los curadores de los herbarios USM, MOL, HAO, HUT, CPUN y PRG por las facilidades brindadas durante la revisión de sus colecciones. A Hernando Layza Vera por su colaboración durante los trabajos de campo en el departamento de La Libertad y Cajamarca. A Asunción Cano y demás investigadores del Laboratorio de Florística de USM por colaborar en los últimos años con información y registros. A Oliver Whaley y Alfonso Orellana por compartir información sobre las lomas del departamento de Ica. A Carlos Vargas y Wilfredo Mendoza por las correcciones y mejoras en el texto.

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## SEM AND PHYLOGENETIC ANALYSIS OF NATURALIZED AND CULTIVATED *EPIDENDRUM* IN HAWAII

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**ABSTRACT.** Naturalized populations of *Epidendrum* L. are found on a rocky hillside in Nuuanu-Pali and Olomana in the Koolau Mountains of Oahu, Hawaii. Scanning electron micrographs were taken to observe polymorphism among the pollinia, petals, leaves, and root tips of two *Epidendrum* specimens (one naturalized specimen from Nuuanu-Pali and one cultivated specimen in the greenhouse). SEM images of pollen from the naturalized *Epidendrum* revealed a length of 830.31  $\mu\text{m}$  and a width of 462.58  $\mu\text{m}$ . Pollen length from the cultivated cultivar, by comparison, was 724.60  $\mu\text{m}$  and the width 276.17  $\mu\text{m}$ . Differing cell structures on the lower surface of the petals were also observed. Polyhedral concave cells with numerous fossae (pits) were seen on the naturalized cultivar and elongated flattened cells on the cultivated one. Transsections of the leaf of the naturalized specimen were much thinner (546.33  $\mu\text{m}$ ) compared to the thickness of the cultivated cultivar leaf (1505.83  $\mu\text{m}$ ), which contained more spongy parenchyma cells. A thinner root tip (1094.19  $\mu\text{m}$ ) was seen in the naturalized cultivar, as opposed to 1636.34  $\mu\text{m}$  in the cultivated specimen. To compare relationships between these two specimens along with ten other unknown *Epidendrum* cultivars, we sequenced the plastid *trnL-F* gene region and conducted parsimony analysis among the naturalized *Epidendrum* from Nuuanu-Pali. At least six changes separated these specimens into two clades. Shorter and longer plastid simple sequence repeats (cpSSR) from the *rps16-trnK* region support separation of the five *Epidendrum* genotypes evaluated into these two groups, including a naturalized *Epidendrum* from Olomana.

**KEY WORDS:** *Epidendrum*, scanning electron microscopy, *trnL-F*, *rps16-trnK*

*Epidendrum* L. species are native to the tropical Americas. They are found from sea level to 10,000 feet elevation, growing as terrestrials, epiphytes or lithophytes. The reed-stem *Epidendrum radicans* Pav. ex Lindl. bears racemes in many shapes and shades of color, such as red, orange, purple or white. Some are marked with yellow on the base of the labellum (Phillips & Hill 1998).

Luer (1975) mentioned that the hybrid *E. ×obrienianum* (*jamiesonis* x *radicans*) registered by Veitch in 1888 in England was brought to Hawaii and subsequently naturalized there. Jan Goo (orchid grower) made many hybrids in the 1940s in Hawaii using *E. radicans*, *E. ibaguense* Kunth, *E. ellipticum* Graham, *E. arachnoglossum* Rehb.f. ex André, and *E. cinnabarinum* Salzm. ex Lindl. Backcrosses were also made using primary hybrids including *E. ×obrienianum* (Sander & Wreford 1961). Unfortunately, many of his hybrids today that are commonly grown as garden plants or in greenhouses

have lost their registered names (Rose & Rose 2005).

Scanning electron microscopy and molecular phylogenetic studies were conducted at the University of Hawaii - Manoa, University of Hawaii - Windward Community College, and the Royal Botanic Gardens, Kew, from summer 2006 to fall 2012 to determine the identity of the wild/naturalized *Epidendrum* accessions from Nuuanu-Pali and Olomana on Oahu and compare them to their cultivars commonly grown in the greenhouse.

### Materials and methods

**Scanning electron microscopy (SEM).** – An *Epidendrum* specimen was collected from a rocky hillside in Nuuanu-Pali, Oahu. It had many roots along the stem. The leaf was 1.6 cm wide  $\times$  6 cm long. The flower was 3 cm wide  $\times$  3.3 cm high (WCC voucher # 934). The greenhouse-grown cultivar at Windward Community College had a leaf 2 cm wide  $\times$  10 cm long and a flower 2.6 cm wide  $\times$  3 cm high (WCC voucher # 935).

Pollinia, transverse sections of 2 mm-wide petals and leaves, and transverse sections of 5 mm-long root tips from wild-collected and cultivated accessions were fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4, for 2 hr at 25 C. Tissues were washed twice in 0.1 M sodium cacodylate buffer for 10 minutes each and then post-fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 hr. Specimens were then dehydrated in a graded ethanol series as follows: 30% (2x for 5 min each), 50% (2x for 5 min each), 70% (3x for 5 min each), 85% (3x for 5 min each), 95% for 5 min, 98% for 10 min, and 100% before being stored in the refrigerator overnight.

Tissues were then critical-point-dried in a SAMDRI-795 machine. Dried tissues were each mounted on carbon-conductive tape secured on metal stubs for gold-palladium coating using a Hummer 6.2 Sputter Coater. Scanning electron micrographs were taken with a Hitachi S-800 field emission SEM.

*Plastid DNA amplification and sequencing.* – Total genomic DNA was extracted from silica gel-exsiccated leaves of 12 accessions using the modified Doyle and Doyle (1987) protocol. Samples included one naturalized *Epidendrum* collected from Nuuanu-Pali and 11 cultivated *Epidendrum* plants obtained from four greenhouses (Table 1). Samples were photographed, and living specimens were grown in the climate-controlled greenhouse at Windward Community College as voucher specimens. Target loci *trnL-F* were amplified in 25 µL volumes using standard polymerase chain

reaction (PCR) protocols that included the addition of bovine serum antigen (BSA). Primers *trnLc* (5'-CGAAATCGGTAGACGCTACG -3') and *trnLf* (5'-ATITGAAGTGGTGACACGAG -3') were used to amplify and sequence *trnL-F* (Taberlet *et al.* 1991). Following sequencing at the Jodrell Laboratory at the Royal Botanic Gardens, Kew, electropherograms were edited using Sequencher 3.1 software (GeneCode Corp., Ann Arbor, Michigan, USA). The resulting *trnL-F* data were analyzed and a dendrogram prepared to include an *Epidendrum ibaguense* nucleotide database available from GenBank for comparison.

*Plastid simple sequence repeats (cpSSR).*– Plant DNA was isolated using NucleoSpin® Plant II mini spin columns (Macherey-Nagel, Inc., Bethlehem, Pennsylvania). As previously described by Pinheiro *et al.* (2009), primers Epcp-02-Forward 5'-TTCTTGCTTCTTTTGTGGA -3' and Epcp-02-Reverse 5'- ATTTGTTTGATACGCCATTG -3' were used to amplify the plastid locus Epcp-02 (*rps16-trnK*) from the five genotypes. Accuzyme DNA polymerase (Bioline, Taunton, Massachusetts), which has 3'-5' proofreading exonuclease activity, was used to reduce the rate of erroneous base insertions associated with non-proofreading DNA polymerase.

PCR products were ligated directly from the PCR reactions into the vector PCR-Blunt (Life Technologies, Grand Island, New York). Ligation reaction products were used to transform chemically competent *E. coli* (One Shot TOP10 cells; Life Technologies, Grand Island, New York). Plasmid

TABLE 1. List of silica gel-exsiccated leaves from 12 *Epidendrum* accessions.

No.	Plant labels	Leaf fwt (g)	Flower color and size (cm)	Leaf size (cm) and stem color
1	<i>Epi. Clem (Epi. C)</i>	6	dull orange, yellow callus. W: 2.5, L: 3	W: 2, L: 6.5
2	<i>Epi. Inge (Epi. I)</i>	6	purplish red, yellow callus. W: 2.6, L: 3	W: 2, L: 10
3	<i>Epi. Clay 6</i>	6	bright red, yellow callus, red dots. W:2.6, L: 3	W: 1.6, L: 6.2
4	<i>Epi. Clay 3</i>	6	pure yellow. W: 2.2, L: 2.4. Pedicel: 2.7	W: 4.5, L: 1.8. Purple stem
5	<i>Epi. Clay 7</i>	6	pure yellow. W: 2.2, L: 2.4. Pedicel: 2.5	W: 2, L: 7.5
6	<i>Epi. Clay 1</i>	6	brownish orange. W: 2.9, L: 3	W: 2, L: 4.4
7	<i>Epi. 'Pali' (Epi. P)</i>	6	bright red, yellow callus, red dots. W:3, L:3.3	W: 1.6, L: 6
8	<i>Epi. Clay 2</i>	6	possibly yellow/red	W: 1.9, L: 5.7
9	<i>Epi. Clay 8</i>	6	possibly red/orange	W: 1.3, L: 6
10	<i>Epi. Clay 5</i>	4.545	possibly red	W: 1.3, L: 4.6
11	<i>Epi. Clay 4</i>	3.468	possibly red/purple	W: 1.2, L: 4.5
12	<i>Epi. small white (Epi. sm12)</i>	6	white w/light purple base. W: 1.5, L: 1.7	W: 1.9, L: 7.5



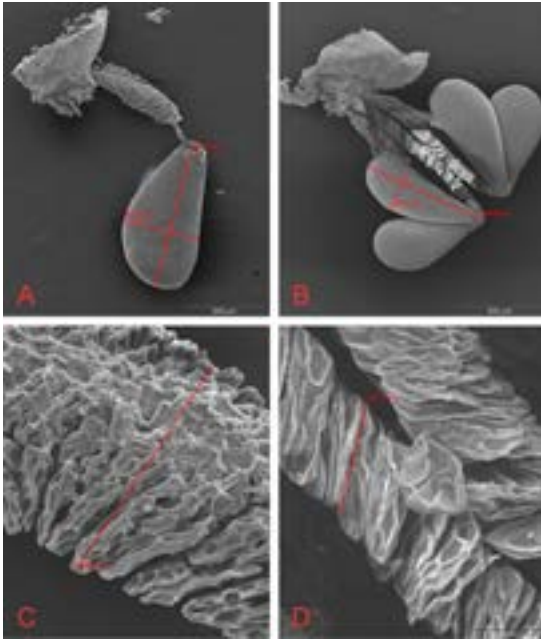


FIGURE 1. SEM images of pollinia (60x) and stipes (500x) of *E. P* and *E. I*. A. Pollen size of *E. P* (830.31  $\mu\text{m}$  long and 462.58  $\mu\text{m}$  wide); B. Pollen size of *E. I* (724.60  $\mu\text{m}$  long and 276.17  $\mu\text{m}$  wide); C. Stipe of *E. P* (deeply folded and rugose, 178.81  $\mu\text{m}$  wide); D. Stipe of *E. I* (84.15  $\mu\text{m}$  wide).

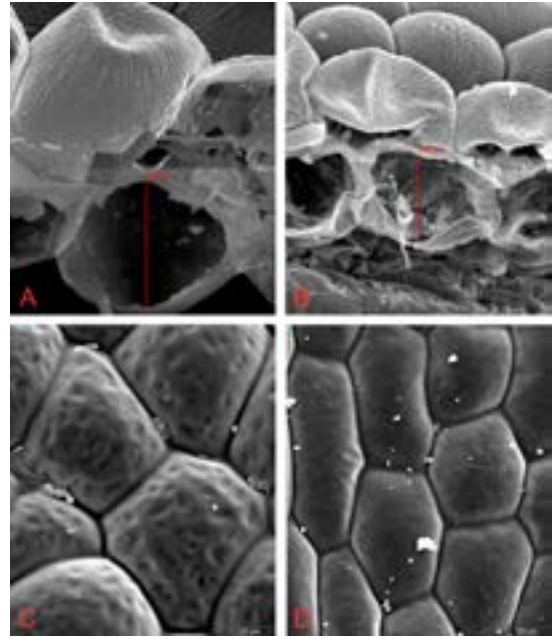


FIGURE 2. SEM images of the upper layer (1,300x) and the lower layer (800x) of petals of *E. P* and *E. I*. A. Bulbous cells on the upper layer of the petal of *E. P* (36.10  $\mu\text{m}$  high); B. Bulbous cells on the upper layer of the petal of *E. I* (24.85  $\mu\text{m}$  high); C. Polyhedral convex cells with numerous fossae seen on the lower layer of the petal of *E. P*; D. Elongated flattened cells on the lower layer of the petal of *E. I*.

DNA purification was performed using NucleoSpin® Plasmid mini spin columns (Macherey-Nagel, Inc., Bethlehem, Pennsylvania). Sequence analysis was conducted at University of Hawaii's Advanced Studies in Genomics, Proteomics and Bioinformatics using a 3700 xl DNA Analyzer (Applied Biosystems, Foster City, California).

### Results and discussion

Scanning electron microscopy of pollen from the naturalized *Epidendrum* from Nuuanu-Pali (*E. P*) showed a length of 830.31  $\mu\text{m}$  and a width of 462.58  $\mu\text{m}$  (Fig. 1A). Length of the pollen from the cultivated cultivar (*E. I*), by comparison, was 724.60  $\mu\text{m}$ , and the width was 276.17  $\mu\text{m}$  (Fig. 1B). A more deeply folded and rugose stipe was observed in *E. P* (Fig. 1C). Its width was also greater (178.81  $\mu\text{m}$ ), compared to 84.15  $\mu\text{m}$  in *E. I* (Fig. 1D).

Similar bulbous cell structures were found on the upper layer of petals from both *E. P* and *E. I*. The heights of *E. P* and *E. I* bulbous cells were 36.10  $\mu\text{m}$

and 24.85  $\mu\text{m}$ , respectively (Fig. 2A, B). Differing cell structures occurred on the lower surface of the petals. Polyhedral convex cells with numerous fossae (pits) belong to *E. P* (Fig. 2C), whereas elongated flattened cells are seen on *E. I* (Fig. 2D).

The two cultivars showed no detectable differences in leaf and/or root tip cell structures other than a much thinner transverse section of *E. P* leaf tissue (546.33  $\mu\text{m}$ ) (Fig. 3A) compared to a 1505.83  $\mu\text{m}$  thickness in *E. I* (Fig. 3B). Differences in the thickness of leaf tissues were determined by differences in the size of the cells as seen in each of their vascular bundles. Vascular bundle heights of *E. P* and *E. I* were 119.68  $\mu\text{m}$  and 189.26  $\mu\text{m}$ , respectively (Fig. 3C, D). A thinner root tip (1094.19  $\mu\text{m}$ ) was also observed in the naturalized *E. P*, as opposed to the thicker root tip (1636.34  $\mu\text{m}$ ) of the cultivated *E. I* (Fig. 4A, B). The sizes of their respective parenchyma cells are 21.76  $\mu\text{m} \times 23.03 \mu\text{m}$  for *E. P* and 41.11  $\mu\text{m} \times 31.90 \mu\text{m}$  for *E. I*. The greater thickness of leaf and root tissues of the cultivated cultivar might be attributed to the abundant and continuous water received during

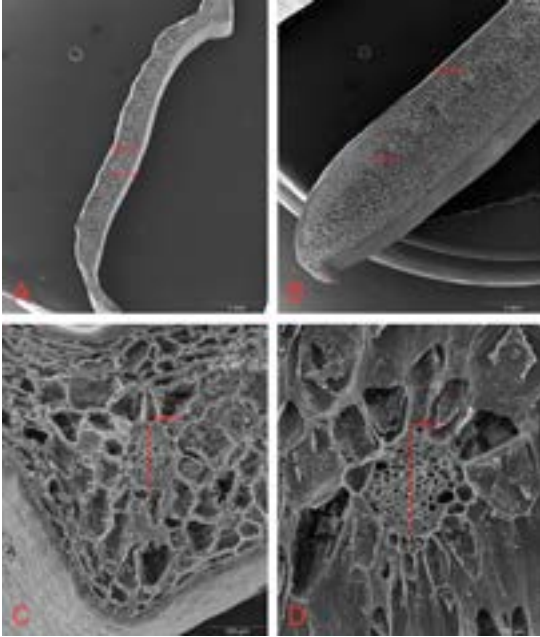


FIGURE 3. SEM images of x-sections of leaves (20x) and midribs (200x) of *E. P* and *E. I*. A. Leaf thickness of *E. P* (546.33  $\mu\text{m}$ ); B. Leaf thickness of *E. I* (1505.83  $\mu\text{m}$ ); C. Vascular bundle height of *E. P* (119.68  $\mu\text{m}$ ); D. Vascular bundle height of *E. I* (189.26  $\mu\text{m}$ ).

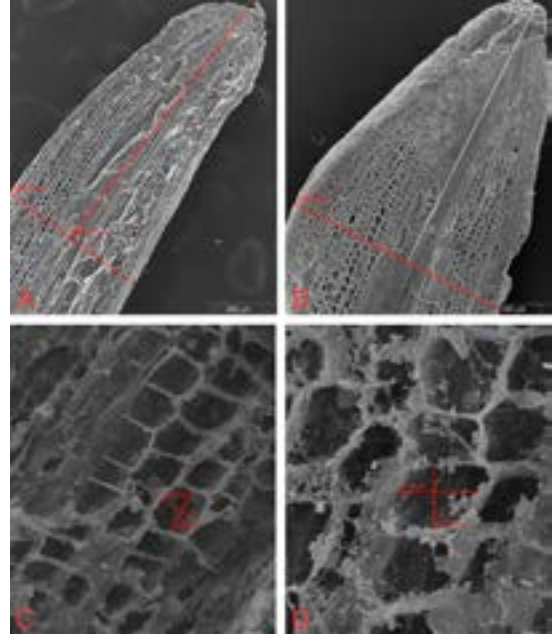


FIGURE 4. SEM images of 5 mm longitudinal section of root tips (50x) and parenchyma cells (700x) of *E. P* and *E. I*. A. Root tip tissue of *E. P* (1094.19  $\mu\text{m}$ ); B. Root tip tissue of *E. I* (1636.34  $\mu\text{m}$ ); C. Parenchyma cell size of *E. P* (21.76  $\mu\text{m}$  x 23.03  $\mu\text{m}$ ); D. Parenchyma cell size of *E. I* (41.11  $\mu\text{m}$  x 31.90  $\mu\text{m}$ ).

its growth in a climate-controlled greenhouse equipped with a sprinkler system.

Morphological differences in cell structures found on the stipes and lower surfaces of petals indicate that the naturalized *E. P* and the cultivated *E. I* might have genetically divergent origins. A single *trnL-F* tree is shown in Figure 5. Parsimony analysis among the naturalized *E. P* and the other 11 greenhouse-cultivated *Epidendrum* accessions showed that at least six changes separate these specimens into two separate groups. *Epidendrum* Clay 1, *E. Clay 3*, *E. Clay 7*, *E. Clem*, *E. P*, and *E. sw12* belong to one group of 4 units in length. *Epidendrum* Clay 2, *E. Clay 4*, *E. Clay 5*, *E. Clay 6*, *E. Clay 8*, and *E. I* belong to another group of 2 units in length. The naturalized *E. P* belongs to the first group, and the cultivated *E. I* belongs to the second group. Sequence data seem to support the morphological dissimilarities seen in the SEM images of these two corresponding genotypes. Within the first group, *E. Clay 1*, *E. Clay 3*, and *E. sw12* are differentiated into subgroups of 1 unit in length. These three specimens are closely related *Epidendrum*

hybrids of white-yellow flowers. In the second group, *E. Clay 5* is differentiated further into a subgroup of 1 unit in length. It also appears to be a hybrid, with flower color undetermined at this time.

Locus *Epcp-02 (rps16-trnK)* was previously reported to contain a microsatellite in *Epidendrum* species that varies in the number of cytosines from  $C_8$  to  $C_{11}$ . Interestingly, in this study a range from  $C_8$  in *E. IB*, to  $C_{16}$  in *E. O* was observed. Using this simple sequence repeat (SSR), *E. I* and *E. IB* (shorter mono tandem repeats  $C_{10}$  and  $C_8$ , respectively), could be distinguished from the longer mono tandem repeats  $C_{16}$  (*E. O*) and  $C_{14}$  (*E. RP*, and *E. C*) (Fig. 6). *Epidendrum I* and *E. IB* have the same single-nucleotide polymorphism (SNP) guanines at positions 65 and 63, respectively, which distinguish these lines from the other genotypes. This finding supports categorization of the genotypes based on long ( $C_{14-16}$ ) and short ( $C_{8-10}$ ) mono tandem repeats. *Epidendrum I* can be distinguished from *E. IB* as well the other genotypes by cytosine at base pair position 204.

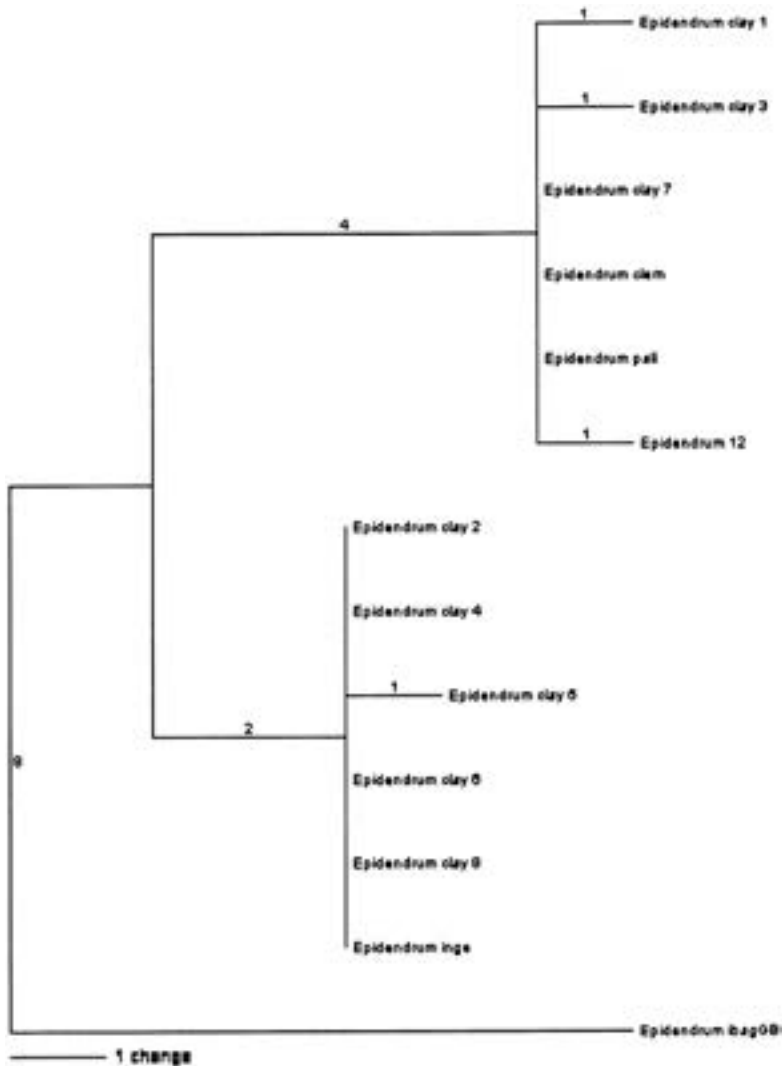


FIGURE 5. Most-parsimonious tree obtained in a cladistic analysis of plastid (*trnL-F*) DNA sequences plus one structural character of the root. Numbers above branches are number of changes.

In combination, the SSR and polymorphism associated with Locus Epcp-02 (*rps16-trnK*) can be used to distinguish related *Epidendrum* genotypes. The evaluation of additional genotypes will be required to determine if the cytosine at base pair 204 of *E. I* is unique to this genotype and if long (C14 – 16) and short (C8 – 10) SSRs correlate with established morphological differences that may distinguish these groups. Further study will be done when the following specimens (*E. radicans*, *E. evectum*, and *E. ×obrienianum*) become available.

ACKNOWLEDGEMENTS. The corresponding author wishes to thank the USDA – NIFA (United States Department of Agriculture – National Institute of Food and Agriculture) for research and travel funding to present an oral presentation at the Fourth Scientific Conference on Andean Orchids in Guayaquil, Ecuador, in 2012. We thank Tina Carvalho from the Biological Electron Microscope Facility, the Research Corporation of the University of Hawaii, Manoa, for assisting in the preparation of specimens for scanning electron micrographs; Dr. Kenneth Cameron, Director of the Wisconsin State Herbarium, for exporting the 12 *Epidendrum* silica gel-dried leaves to the Jodrell Laboratory

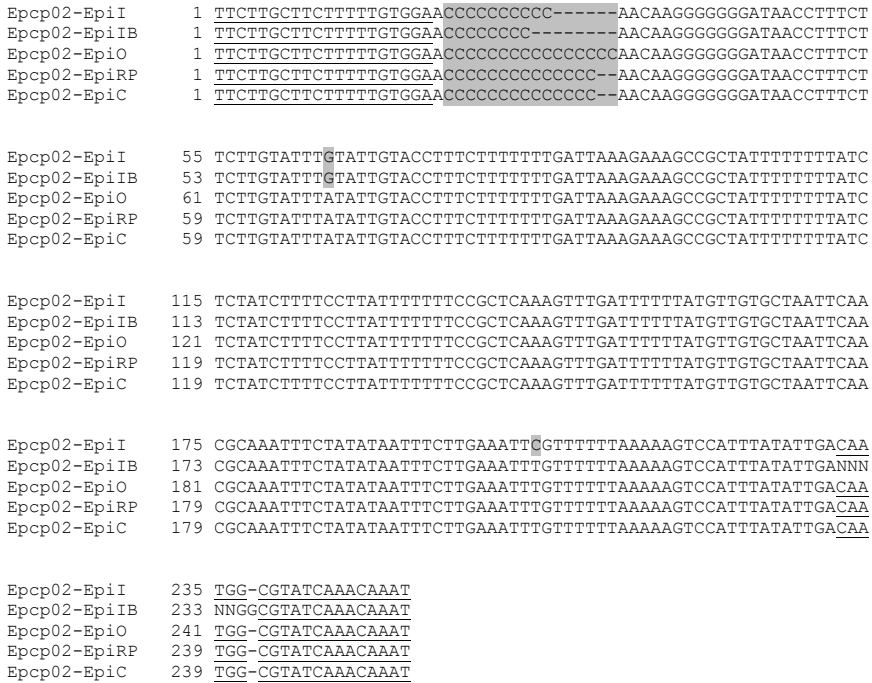


FIGURE 6. Sequence alignment of the chloroplast locus Epcp-02 (*rps16-trnK*) from four cultivated cultivars: *Epidendrum Inge* (E. I), *E. ibaguense* (E. IB), *E. Red Purple* (E. RP), *E. Clem* (E. C), and a naturalized *Epidendrum* from Olomana (E. O). Forward and reverse primer sequences are underlined.

at the Royal Botanic Gardens, Kew. Special thanks are extended to Clayton Akatsuka, Clem Roldan, and Scot Mitamura for donations of cultivated plants and to Nyan Stillwell for collecting the naturalized *Epidendrum* from Olomana and helping in the Windward Community College laboratory.

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**TULASNELLA IRREGULARIS (BASIDIOMYCOTA: TULASNELLACEAE)  
FROM ROOTS OF *ENCYCLIA TAMPENSIS* IN SOUTH FLORIDA,  
AND CONFIRMATION OF ITS MYCORRHIZAL SIGNIFICANCE  
THROUGH SYMBIOTIC SEED GERMINATION**

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**ABSTRACT.** Epiphytic orchids remain understudied with respect to their obligate mycorrhizal relationships – a key component of the integrated conservation model. Existing studies have revealed that these plants, like their terrestrial counterparts, commonly associate with ubiquitous basidiomycetes (*e.g.*, Tulasnellaceae); however, few studies have verified their physiological role(s). Two strains of mycorrhizal fungi (UAMH 11541, UAMH 11543) were isolated from roots of an epiphytic orchid in south Florida, *Encyclia tampensis*; one was acquired from a seedling and one from a mature specimen. Seeds of four epiphytic taxa were subsequently inoculated (separately) with both fungal strains *in vitro*: *E. tampensis*, *Epidendrum amphistomum*, *Epidendrum nocturnum*, and *Prosthechea cochleata*. More than one-third of inoculated *E. tampensis* and *E. nocturnum* seeds developed leaves in total darkness after 100 days. No significant differences were detected between the two strains on germination, nor any interaction between fungus and seed source (ANOVA,  $\alpha = 0.05$ ). Using ITS amplification and sequencing, both strains were identified as the teleomorph, *Tulasnella irregularis* (Basidiomycota: Tulasnellaceae), and both were genetically identical with a high (98%) degree of certainty. Thus, symbiotic germination and ITS sequencing results are in agreement that both strains are indeed the same fungus. This paper is meant to shed additional light into epiphytic orchid-fungal interactions and highlights the need to identify, test (through symbiotic germination) and safeguard mycorrhizal fungi necessary for conservation.

**RESUMEN.** Las relaciones micorrízicas obligadas de las orquídeas epífitas – un componente clave en el modelo integrado de conservación – siguen desconocidas. Los estudios existentes han revelado que estas plantas, así como sus equivalentes terrestres, se asocian normalmente con basidiomicetos ubicuitos (*e.g.* Tulasnellaceae); sin embargo pocos estudios han verificado su papel fisiológico. Dos cepas del hongo micorrítico (UAMH 11541, UAMH 11543) fueron aisladas de las raíces de una especie de orquídea epifítica del sur de Florida, *Encyclia tampensis* – una obtenida de una plántula joven y la otra obtenida de una planta madura. Las semillas de cuatro taxones epífitos fueron inoculadas por separado con los dos hongos aislados *in vitro*: *E. tampensis*, *Epidendrum amphistomum*, *Epidendrum nocturnum* y *Prosthechea cochleata*. Más de un tercio de las semillas de *E. tampensis* y *E. nocturnum* (ambos inoculados) desarrollaron hojas cuando fueron mantenidos en total obscuridad por más de cien días. No se detectaron diferencias significativas entre las dos cepas del hongo con respecto a la germinación y la interacción entre el hongo y las semillas procedentes de diferentes fuentes (ANOVA,  $\alpha = 0.05$ ). Usando amplificación y secuenciación ITS, ambas cepas fueron identificadas como teleomorfos, *Tulasnella irregularis* (Basidiomycota: Tulasnellaceae) y ambas resultaron genéticamente idénticas con un elevado grado (98%) de seguridad. De esta manera, tanto la germinación simbiótica así como los resultados de la secuenciación ITS concuerdan en identificar con certeza ambas cepas como el mismo hongo. Este artículo trata de elucidar las interacciones entre orquídeas epífitas y hongos micorríticos y también subraya la necesidad de identificar, comprobar (a través de germinación simbiótica), y preservar los hongos micorríticos necesarios para fines de conservación.

**KEY WORDS:** Conservation, epiphytic orchids, mycorrhizal relationships, physiology, ITS sequencing, Basidiomycota, *Tulasnella irregularis*, *Encyclia tampensis*, *Epidendrum*

Orchids occur naturally on all vegetated continents (Dressler 1981), but about three-quarters of all known species (73%) exist as epiphytes within the tropics (Atwood 1986). Of the family's estimated 17,000-35,000 species worldwide (Atwood 1986; Dressler, 1993), orchid diversity is richest in the New World (Cribb *et al.* 2003). In light of accelerated destruction of the world's tropical forests in this century and the last, many orchid species face almost certain extinction unless effective strategies aimed at their long-term conservation are swiftly implemented. For orchids in particular, this will be a daunting task given the high degree to which these plants rely on other biotic agents (*e.g.*, pollinators, mycorrhizal fungi) for their reproduction and survival needs. As a result, integrated conservation — blending ecological/genetic studies with *ex situ* and *in situ* research — has emerged as a more complete, inclusive approach to orchid conservation (see Swarts & Dixon 2009), evidenced by studies in Australia (Swarts 2007) and North America (Stewart 2007).

Compared to temperate terrestrial orchids, tropical epiphytes remain understudied with respect to their obligate mycorrhizal relationships (Otero *et al.* 2007), a key component of the integrated model. However, a growing number of studies have emerged in recent years that document the identity of mycorrhizal fungi associated with epiphytic orchids worldwide using DNA and TEM methods (*e.g.*, Aggarwal *et al.* 2012; Ma *et al.* 2003; Martos *et al.* 2009; Pereira *et al.* 2003, 2005; Roy *et al.* 2009; Herrera *et al.* 2010; Kottke *et al.* 2010; see review by Dearnaley *et al.* 2013). Like their temperate terrestrial counterparts, epiphytic orchids (so far) appear to associate commonly with ubiquitous basidiomycetes assignable to Ceratobasidiaceae, Tulasnellaceae and Sebaciniales. While interesting as this new information may be, most studies have targeted only mature plants rather than seedlings, and most have merely identified these fungi without verifying their physiological role(s). For integrated conservation to be successfully applied to the epiphytes, studies must also isolate, identify and preserve mycorrhizal fungi, including those from early growth stages (protocorms, seedlings), but this may be viewed as problematic given that locating diminutive seedlings on arboreal substrates is not always easy or practical. To facilitate the recovery of

protocorm stages, a seed-baiting technique modified for epiphytic orchids (Zettler *et al.* 2011) may hold some promise in capturing fungi that initiate the germination processes *in situ*. Though access to tree limbs may be physically challenging, locating tiny leaf-bearing seedlings on arboreal substrates is relatively easy once practiced with a well-trained eye. Seedlings subsequently recovered may likewise harbor mycorrhizal strains that play a key role in the orchid's growth and development.

In nature, all orchids are thought to have a critical need for mycorrhizal fungi as a carbon source to propel orchid growth stages to completion. For horticultural purposes, the use of fungi to germinate orchid seeds *in vitro* (= symbiotic seed germination) has been largely ignored for the epiphytes because of the ease with which these plants can be grown on asymbiotic (carbon-based) media, unlike temperate (hardy) terrestrials that are notorious for having fastidious germination requirements (Rasmussen 1995). As a propagation tool, symbiotic germination (Clements *et al.* 1986; Dixon 1987) not only appears to have merit for epiphytic orchids (*e.g.*, Aggarwal *et al.* 2012; Zettler *et al.* 2007; see Bayman 2012) but can also be used to verify the physiological role(s) of peloton-forming fungi. Although *in vitro* outcomes may or may not reflect what actually occurs *in situ*, the use of symbiotic germination for this purpose does provide some baseline for assessing mycorrhizal fungi for the purposes of conservation when viewed in the proper context.

In this paper, we describe the isolation of two strains of mycorrhizal fungi from roots of an epiphytic orchid in south Florida, *Encyclia tampensis* (Lindl.) Small, spanning two growth stages (leaf-bearing seedling, mature plant). The use of ITS amplification and sequencing was carried out to identify these strains, and *in vitro* symbiotic seed germination was applied to verify their physiological role. Seeds from three other epiphytic taxa (*Epidendrum amphistomum* A.Rich., *E. nocturnum* Jacq., *Prosthechea cochleata* (L.) W.E.Higgins) from the same region (Collier County, Florida, USA) were also inoculated to test for fungal specificity. The goal of this study is to augment long-term conservation of *E. tampensis* and other epiphytic orchids by emphasizing mycorrhizal fungus recovery, use, assessment, and preservation.





FIGURES 1-4. *Encyclia tampensis* from the Florida Panther National Wildlife Refuge. 1. Close-up of an *E. tampensis* flower. Though still common, this species is commercially exploited for its appealing floral display. Scale bar = 1 cm. 2. Young seedlings of *E. tampensis* growing on the host tree, *Quercus virginiana* Mill., beneath a mature orchid. The narrow, pale green strap leaf and pseudobulb on each seedling are indicative of *E. tampensis*. Scale bar = 2 cm. 3. A tiny *E. tampensis* seedling growing in close proximity to larger seedlings seen in Fig. 2. Scale bar = 2 cm. 4. Subsequent removal of the seedling in Fig. 3 showing roots that yielded *Tulasnella irregularis* (UAMH 11543).

### Material and methods

*Orchid material and study site* — Roots and mature seeds of *E. tampensis* (Fig. 1, 2) were collected from the Florida Panther National Wildlife Refuge

(FPNWR) located in remote Collier County, Florida (USA) within the Big Cypress Basin eco-region of south Florida. Roots were collected from a small, leaf-bearing seedling (Fig. 3, 4) and a mature (flowering) specimen affixed to the SW-facing bark of *Quercus*

*virginiana* Mill. (Fagaceae) on 29 June 2011. The seedling was located ca. 0.5 m below the mature donor plant and was one of ca. 20-30 seedlings (Fig. 2) visible in various growth stages along the length of the host tree's bark. Their identification as *E. tampensis* was based on the narrow, pale green, rigid strap leaves, and characteristic ovoid pseudobulbs subtending the leaves on the larger seedlings. Although *E. tampensis* is occasionally found in wetter habitats (cypress domes) in association with other epiphytic taxa in the FPNWR, it is the only leaf-bearing species to occupy the drier upland sites suitable for *Q. virginiana*. Using a small spatula, the donor seedling with two roots (Fig. 4) was gently lifted from the surface of the substrate and placed into a pre-sterilized glass vial. One 10 cm-long root from the mature plant was likewise gently lifted from the substrate using a scalpel and placed into a separate pre-sterilized glass vial. Both sets of roots were then transported to the laboratory and refrigerated (6° C) for one week leading up to fungal isolation.

Seeds from four epiphytic orchid taxa were collected from Collier County, Florida, for use in symbiotic germination experiments: *E. tampensis*, *E. amphistomum*, *E. nocturnum*, and *Prosthechea cochleata*. Although *E. tampensis* is considered a common species, it is commercially exploited for its showy floral display (Fig. 1). The other three taxa are listed as state-endangered (Brown 2005). Three seed sources were collected from *E. tampensis* (S194, S195, S196) and one source for each of the other three taxa:

*E. amphistomum* (S197), *E. nocturnum* (S20), and *P. cochleata* (S177) (Table 1). Mature capsules that appeared to be in the act of opening naturally were collected and placed over CaSO<sub>4</sub> desiccant (Drierite, W.A. Hammond Co., Xenia, Ohio, USA) in separate vials for transport to the laboratory. Within seven days of collection, capsules were placed over fresh Drierite desiccant at ambient temperature until seeds were thoroughly dry. Seeds were then removed by gently tapping the outer surface of the capsule over aluminum foil, then placed in sealed glass vials and stored at -7° C in darkness until use.

*Fungal isolation, initial identification and preservation* — Mycorrhizal fungi were isolated from the root cortical region using standard procedures (e.g., Currah *et al.* 1987, 1990; Richardson *et al.* 1993; Zettler *et al.* 2003). Roots were surface-sterilized for 1 min. in a solution of 5% absolute ethanol (EtOH), 5% Clorox bleach (5.25% NaOCl; Clorox Co., Oakland, California, USA) and 90% sterile DI water, followed by two 1 min. rinses in sterile DI water. Clumps of macerated cortical cells containing pelotons were immersed in Fungal Isolation Medium (FIM) containing streptomycin sulfate (Clements & Ellyard 1979) and incubated at ambient temperature. After 1-4 days, hyphal tips that emerged from the cortical region and/or pelotons were subcultured to potato dextrose agar (PDA, Difco™, Becton, Dickinson and Co., Sparks, Maryland, USA) using a sterile scalpel and dissection microscope. Orchid mycorrhizal strains were initially distinguished

TABLE 1. Six seed sources from four epiphytic orchid species utilized in symbiotic germination experiments. All seeds were derived from mature capsules on specimens that grew naturally in Collier Co., FL, and all were obtained from the Florida Panther NWR with the exception of S20.

Seed Source	Orchid	Date collected	Notes
S20	<i>Epidendrum nocturnum</i>	1 June 2002	Fakahatchee Strand, ca. 15 km S of FPNWR
S177	<i>Prosthechea cochleata</i>	14 March 2009	McBride's Pond (cypress dome)
S194	<i>Encyclia tampensis</i>	20 November 2011	Both S194 and S195 from two separate plants ca. 1 km SE of McBrides' Pond
S195	<i>Encyclia tampensis</i>	20 November 2011	See above
S196	<i>Encyclia tampensis</i>	6 January 2012	Dry site ca. 100 m E of McBride's Pond
S197	<i>Epidendrum amphistomum</i>	28 June 2011	McBride's Pond (cypress dome)



from common molds using previously published descriptions (Currah *et al.* 1987; 1990; Richardson *et al.* 1993; Zettler *et al.* 2003). Fungi that yielded cultural characteristics on PDA assignable to the anamorphic form-genus *Rhizoctonia* and *Epulorhiza* in particular (Currah *et al.* 1997a; Moore 1987), were stored at Illinois College under refrigeration (4° C) in darkness on PDA slants in screw-cap tubes for eventual use in symbiotic germination experiments. Subcultures of these *Epulorhiza* strains were also deposited in the University of Alberta (Canada) Microfungus Collection and Herbarium (UAMH) for permanent safekeeping and future reference as: UAMH 11541, UAMH 11542, and UAMH 11543.

*Molecular identification of fungi* — Two of the fungi deposited into UAMH, one from the *E. tampensis* seedling (UAMH 11543) and one from the mature plant (UAMH 11541), were identified further via sequencing of the ITS regions of ribosomal DNA. To facilitate DNA isolation, colonies of pure fungus cultures were grown on liquid media (potato dextrose broth, Difco™) on a shaker at ambient temperature until harvesting, ca. 1 month after inoculation. DNA was isolated from liquid fungal cultures using the Omega EZNA Fungal DNA Mini Kit protocol for fresh/frozen samples (Omega Biotek, Doraville, Georgia, USA). The ITS regions of DNA isolates were amplified using primers ITS1-OF-T and ITS4-OF (Taylor & McCormick 2008). The reactions contained 1x EZNA Taq Buffer, 0.1 mM dNTPs, 25 pmoles ITS1-OF-T, 25 pmoles ITS4-OF, 12.5 units Taq polymerase and 5 µl of DNA sample. The amplification was performed in a programmable thermal cycler (Labnet, Edison, New Jersey, USA) programmed for 45 cycles. Each cycle consisted of 94°C for 30 sec, 52°C for 30 sec and 72°C for 60 sec. Amplification products were visualized by electrophoresis on 2% agarose gels containing 0.1 mg/ml ethidium bromide.

*In vitro symbiotic seed germination* — The protocol for symbiotic seed germination closely followed the procedure outlined by Zettler *et al.* (2007), except seeds were pipetted directly onto the agar surface and not onto filter paper. Briefly, seeds were surface-sterilized using the same bleach/EtOH rinse described above for fungal isolations, and ca. 50-100 seeds were dispensed onto the surface of an oat-based medium (2.5 g rolled oats, 7.0 g agar, 1 L DI water; Dixon 1987)

within a 9 cm diam. petri plate using a sterile glass pipette. For each of the six seed sources, 10 replicate plates were prepared and inoculated with the seedling-derived fungus (UAMH 11543), and 10 replicate plates received the fungus isolated from the mature orchid (UAMH 11541). Five replicate plates for each of the six seed sources did not receive fungal inoculation and served as controls. To promote evaporation of the suspension droplet and seed/agar contact, petri plates were wrapped in Parafilm “M” (Pechiney Plastic Packaging, Menasha, Wisconsin, USA) ca. 24 hrs after sowing and inoculation. All plates were then wrapped tightly in aluminum foil to exclude light and incubated at ambient temperature (22° C) for 100 days. Using a dissecting microscope, seed germination and seedling development were assessed on a scale of 0-5 where: Stage 0 = no germination; Stage 1 = swollen embryo/production of one or more rhizoids; Stage 2 = embryo enlargement leading to rupture of the testa; Stage 3 = appearance of shoot region (protomeristem); Stage 4 = emergence of first leaf; Stage 5 = leaf elongation. To test the hypothesis that both fungal strains (UAMH 11541, UAMH 11543) were the same fungus, data were analyzed using general linear model procedures multivariate analysis of variance ( $P < 0.05$ ) and mean separation at  $\alpha = 0.05$  by SPSS 12.0 for Windows subprogram (SPSS, Chicago, Illinois, USA). The experiment was repeated once.

## Results and discussion

*Fungal identification* — Root sections of *E. tampensis* harbored a variety of endophytic fungi evident in petri plates containing FIM, most of which were later identified as common saprophytic molds (*e.g.*, *Pestalotia*) on PDA. Of the ubiquitous anamorphic genera known to commonly associate with orchids worldwide, only *Epulorhiza* was evident in the samples. This initial identification was based on the creamy white colony appearance on PDA, coupled with mostly submerged/adnate mycelium with entire margins and relatively slow-growing hyphae (< 0.10 mm/hr) at ambient temperature (Currah *et al.* 1987, 1990; Richardson *et al.* 1993; Zettler *et al.* 2003). Three different strains of *Epulorhiza* were isolated, two of which originated from a different region of the mature plant's root (UAMH 11541, UAHM 11542) and the

third from the seedling root (UAMH 11543). On PDA, all three appeared indistinguishable from one another and closely resembled two other *Epulorhiza* strains isolated previously from orchids in northern Florida: UAMH 9824 from *Spiranthes brevilabris* Lindl. in Levy County (Stewart *et al.* 2003) and UAMH 9203 from *Epidendrum magnoliae* Muhl. (syn. *Epi. conopseum* R.Br.) in Alachua County (Zettler *et al.* 1997). Additional *Epulorhiza* strains have been isolated from Florida orchids including *Habenaria macroceratitis* Willd. in central Florida (Stewart & Kane 2006) and *E. nocturnum* from the Florida Panther NWR (L.W. Zettler, unpubl. data). To what extent these *Epulorhiza* isolates are genetically similar remains unknown, but the use of molecular techniques could be applied to those strains currently in storage at UAMH to resolve this question. In this study, sequencing of the ITS regions of ribosomal DNA revealed that both *Epulorhiza* isolates were assignable to the teleomorph *Tulasnella irregularis* Warcup & Talbot (Basidiomycota, Tulasnellaceae). Not only were these two strains of the same taxon, they both appeared to be genetically identical with a high (98%) degree of certainty. The fact that one strain was isolated from an *E. tampensis* seedling (UAMH 11543) and the other from a mature plant (UAMH 11541) indicates that different growth stages in this orchid are nutritionally tied to this one fungus.

*Seed germination and seedling development* — Both fungal isolates (UAMH 11541, UAMH 11543) facilitated seed germination and seedling development spanning all four orchid taxa *in vitro* 100 days after sowing and inoculation (Table 2). Percent germination exceeded 50% for all three *E. tampensis* seed sources (S194 = >81%; S195 = >50%; S196 = >84%) as well as seeds from *E. nocturnum* (S20 = >68%), whereas seeds from the other two taxa (*P. cochleata*, *E. amphistomum*) failed to develop beyond Stage 2 (Table 2). More than one-third of *E. tampensis* and *E. nocturnum* seeds inoculated with *T. irregularis* initiated and developed leaves (Stage 5) in total darkness. Two seed sources in particular, S20 (*E. nocturnum*) and S194 (*E. tampensis*), resulted in the highest percentage (>64%) of seeds developing to Stage 5 (Table 2). In contrast, seeds sown on the oat-based medium in the absence of fungi (control) largely failed to germinate (<7%). Thus, the presence of *T.*

*irregularis* had a stimulatory effect on seed germination and development. No significant differences were detected between the two strains on seed germination nor any interaction between fungus and seed source (ANOVA,  $\alpha = 0.05$ ). While there was a main effect of seed, both fungal isolates mirrored one another in their ability to germinate and prompt development across seed sources. Thus, symbiotic germination and ITS sequencing results are in agreement that both strains are indeed the same fungus.

*Ecological implications* — Although orchid seeds do contain small traces of food reserves (Rasmussen 1995), the long-held assumption is that mycorrhizal fungi are required as a carbon source to propel orchid seedlings to a photosynthetic stage. For epiphytic orchids, this concept has received more scrutiny given that these plants would have more access to sunlight in the host tree's canopy, compared to seedlings of terrestrials that remain buried underground. In this study, young protocorms of *E. tampensis* and *E. nocturnum* are fully capable of exploiting fungi for their growth and developmental needs, evidenced by advanced growth stages in the absence of light. This outcome supports a similar finding by Zettler *et al.* (1999) for seeds of *E. tampensis* inoculated with a fungus (*Epulorhiza* sp., UAMH 9203) from *E. magnoliae* Muhl. and incubated in darkness for 13 weeks. Likewise, seeds of *E. magnoliae* and *E. nocturnum* also developed leaves in darkness following inoculation with *Epulorhiza* (Zettler *et al.* 1998, 2007). To what extent that protocorms of other epiphytic orchids rely on mycotrophy remains to be determined, but the evidence indicates that this nutritional capability is not restricted to terrestrial orchids, at least under a controlled laboratory setting. The presence of the same strain of *T. irregularis* acquired from a seedling and mature plant alike supports the hypothesis that *E. tampensis* relies on one fungus and also indicates that mycotrophy may continue to play a nutritional role as the orchid matures. This concept also makes sense from the perspective of seedling recruitment and survival. For example, mature orchids that retain mycorrhizal fungi would likely impart a survival advantage to nearby seedlings because the fungus required for germination and seedling development would be more likely to persist on a common substrate. Indeed, Batty *et al.* (2001) and

Table 2. *In vitro* symbiotic seed germination of four native epiphytic orchid species from south Florida (Collier County) using a mycorrhizal fungus (*Tulasnella irregularis*) from *Encyclia tampensis*, 100 days after sowing. Fungal strains UAMH 11543 and UAMH 11541 originated from an *E. tampensis* seedling and mature plant, respectively, that grew naturally on the same host tree (*Quercus virginiana*) within the Florida Panther NWR. No significant differences were detected between the two fungal strains on germination, nor any interaction between fungus and seed source (ANOVA,  $\alpha = 0.05$ ).

	Orchid	Seed Source <sup>1</sup>	$r^2$	# Seeds	# Stage 0	# Stage 1	# Stage 2	# Stage 3	# Stage 4	# Stage 5 <sup>2</sup>	Mean % Germination
<b>UAMH 11543</b>	<i>P. cochleata</i>	S177	10	1,407	1,189	0	218	---	---	---	15.5
	<i>E. amphistomum</i>	S197	9	350	338	0	12	---	---	---	3.4
	<i>E. nocturnum</i>	S20	10	285	79	0	2	0	4	200 (70.2)	72.3
	<i>E. tampensis</i>	S194	10	384	40	0	0	0	5	339 (88.3)	89.6
	<i>E. tampensis</i>	S195	9	504	231	3	13	6	4	231 (45.8)	54.2
	<i>E. tampensis</i>	S196	9	1,507	232	0	3	70	5	636 (42.2)	84.6
<b>UAMH 11541</b>	<i>P. cochleata</i>	S177	10	1,438	1,272	0	166	---	---	---	11.5
	<i>E. amphistomum</i>	S197	9	235	228	0	7	---	---	---	3.0
	<i>E. nocturnum</i>	S20	10	410	128	0	0	0	19	263 (64.1)	68.8
	<i>E. tampensis</i>	S194	10	425	79	0	0	1	14	331 (77.9)	81.4
	<i>E. tampensis</i>	S195	10	597	296	3	6	3	51	238 (39.9)	50.4
	<i>E. tampensis</i>	S196	9	1,194	188	1	3	39	504	459 (38.4)	84.3
<b>Control</b>	<i>P. cochleata</i>	S177	4	709	709	---	---	---	---	---	0.0
	<i>E. amphistomum</i>	S197	2	20	20	---	---	---	---	---	0.0
	<i>E. nocturnum</i>	S20	5	199	199	---	---	---	---	---	0.0
	<i>E. tampensis</i>	S194	5	220	219	0	1	---	---	---	0.5
	<i>E. tampensis</i>	S195	4	153	143	6	4	---	---	---	6.5
	<i>E. tampensis</i>	S196	5	909	908	0	1	---	---	---	0.1

<sup>1</sup>All seeds were obtained from ripe capsules on plants within the Florida Panther NWR except S20 (collected from the Fakahatchee Strand State Preserve).

<sup>2</sup>Number of replicate petri plates for a given treatment; unequal subsample sizes resulted after contaminated plates were discarded.

Growth stages: 0=no germination, 1=swollen embryo/production of rhizoids, 2=embryo enlargement/rupture of testa, 3=appearance of shoot, 4=emergence of first leaf, 5=leaf elongation.

\* Numbers in parentheses reflect the percentage of total seeds that developed to Stage 5.

Diez (2007) both reported higher survival for seedlings in close proximity to the host plant and attributed this to the presence of mycorrhizal fungi. Similarly, Bare (2012) reported that 20 of 22 orchid individuals produced seedlings on the same host tree or within a 1 m radius, implying a mycorrhizal link. This study is the first to confirm the mycorrhizal link between a mature plant and seedling on the same host tree using molecular identification augmented with *in vitro* symbiotic germination. Additional studies are needed to determine if *T. irregularis* is more widespread in *E. tampensis* and other epiphytic orchids.

The question of whether or not orchids display specificity for certain kinds of mycorrhizal fungi has been the subject of interest in recent years, but it is generally assumed that more common and/or widespread orchids exploit a broad range of fungi (= generalists), whereas rare and/or restricted orchids target specific fungal groups (= specialists) (Swarts & Dixon 2009). Few studies (e.g., Otero and Bayman 2009) have attempted to answer this question for epiphytic orchids using *in vitro* symbiotic germination as a tool. In this study, *E. nocturnum* was shown to be capable of utilizing *T. irregularis* acquired from an orchid that typically grows in areas more prone to desiccation (e.g., on *Q. virginiana* host trees). *Encyclia tampensis*, on the other hand, is more widespread throughout Florida where it frequents the drier landscape, but it is also known to occupy more humid habitats (e.g., host trees in cypress domes) in association with other epiphytic orchid species, including *E. nocturnum*. Thus, it is conceivable that *E. nocturnum* could colonize drier sites as an associate of *E. tampensis* if given the opportunity; yet this is often not the case, at least within the Florida Panther NWR. When leaves of these two orchid species are compared, those of *E. tampensis* appear to be better adapted to desiccation (e.g., more narrow, paler, subtended by pseudobulbs), perhaps indicating that *E. nocturnum* may be more restricted by lack of moisture, not by mycorrhizal fungi. Thus far, at least one strain of *Epulorhiza* has been isolated from *E. nocturnum* from a cypress dome in the Florida Panther NWR (L.W. Zettler, unpubl. data). If ITS sequencing verifies that this strain is also *T. irregularis*, this could help explain why both orchids live as associates in wetter habitats, lending further support for moisture as the primary limiting factor in their distribution.

*The future of orchid conservation in south Florida* — About half (106) of North America's orchid species are found in Florida, and half of these species are restricted to the Big Cypress Basin eco-region in the southernmost part of the state (Brown 2005). With two exceptions (*E. tampensis*, *E. magnoliae*), all of North America's epiphytic orchids are confined to this region where subfreezing temperatures are infrequent. Many of these epiphytes (e.g., *Dendrophylax lindenii* (Lindl.) Benth. ex Rolfe, *E. amphistomum*) are also found in the West Indies and even farther south. As such, the Big Cypress Basin eco-region could be viewed as the northern outpost for epiphytic orchid research in the Western Hemisphere. During the past decade, a number of studies have been published involving epiphytic and terrestrial orchids in south Florida, and in the Florida Panther NWR in particular (e.g., Dutra *et al.* 2008, 2009).

Much of this work has been made possible through private, state and federal agencies (e.g., Naples Orchid Society, US Fish & Wildlife Service) that have provided funds as well as facilities. At the Florida Panther NWR, a lab equipped with an autoclave and sterile hood have made it possible to study orchid seed germination requirements *in vitro* as well as *in situ*, and a greenhouse located adjacent to the lab has been used for propagation. In nearby urban areas (Naples, Miami), a strong core of orchid hobbyists provide enthusiasm, some of which is sparked by local and national media coverage (e.g., *USA Today*) and vice-versa. The recently formed North American Orchid Conservation Center (NAOCC), based at the Smithsonian Environmental Research Center in Maryland, is expected to play a key role in orchid conservation this decade, and plans are underway for that organization to adopt the orchid-fungal model. Taken together, the orchids in the Big Cypress Basin eco-region are in a favorable position to receive additional, multi-dimensional study aimed at their long-term conservation. The findings presented in this paper are meant to shed additional light into epiphytic orchid-fungal interactions, and highlight the need to identify, test (though symbiotic germination), and safeguard the mycorrhizal fungi necessary for integrated conservation to be successful.

ACKNOWLEDGMENTS. We warmly thank Larry W. Richardson (U.S. Fish & Wildlife Service) for ongoing and meaningful research collaborations within the Florida Panther NWR and Elizabeth Rellinger Zettler (Illinois College) for statistical analysis. We also acknowledge Andy Stice and Korrie Edwards (Illinois College) for technical assistance, Scott L. Stewart (Morton College), and Lee Hoffman (Native Orchid Restoration Project) for collecting S20 seed, Lynne Sigler (UAMH) for accessioning our fungal isolates, Haleigh Ray (University of Florida) and John McCormick (East Tennessee State University) for field assistance. For photos, we thank Emily Massey and Haleigh Ray (University of Florida) for Fig. 1, and Fig. 2 and 3, respectively. Funding was provided by Illinois College's Student-Faculty Research Committee, Illinois Orchid Society, Prairie State Orchid Society, and U.S. Fish & Wildlife Service, for which we are grateful.

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**Proceedings of the Fourth Scientific Conference on Andean Orchids**

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*SYSTEMATICS***Diversidad y filogenia de *Lepanthes* en Puerto Rico: ¿especiación o inmigración?**

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El género *Lepanthes* tiene cientos de especies, muchas de las cuales tienen distribuciones geográficas muy limitadas. Estas características lo hace un buen modelo para estudiar la diversidad y especiación de orquídeas. Hay nueve especies de *Lepanthes* en Puerto Rico, y todas son endémicas. Este trabajo pretende contestar la pregunta: ¿Estas especies son productos de eventos de especiación dentro de Puerto Rico, o

representan diversas inmigraciones de otras islas? Para contestarla, se estimaron relaciones filogenéticas entre especies de *Lepanthes* basadas en secuencias del ADN del cloroplasto. Hubo muy poca variación entre las especies de Puerto Rico, lo cual sugiere que son productos de especiación reciente y no representan múltiples eventos de inmigración.

**Development of a novel gene silencing method for orchids**R. FRAZER<sup>1</sup>, A. SCHULTE<sup>2</sup>, B. POMAHACOVA<sup>2</sup>, R. VAN VUGT<sup>3</sup> & B. GRAVENDEEL<sup>1\*</sup>

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Orchids contain more than 25,000 species with diverse flowers. Duplications of genes coding for floral shape, color, and symmetry seem to drive this diversity. We are currently developing *Erycina pusilla* as a model for orchid genomics to find further evidence for this hypothesis. The advantages of this species are that it can easily be grown from seed to flowering stage in less than 6 months and that its genome is small (1,475 Gb) and currently fully sequenced in Leiden (NCB Naturalis and LGTC) and

China (BGI). Developing *Erycina pusilla* as a system for functional analysis will add an internationally valued system to the tools currently available for gene silencing of orchids. As a first step we retrieved housekeeping and cell division genes from Illumina HiSeq genome reads for RT PCR and qPCR. Primers for the floral and leaf color genes CHS and PDS were also designed and tested. These will be used in future gene silencing experiments using RNA modulation.

## Diversidad de orquídeas de los bosques altos de la Serranía de los Paraguas, Chocó Biogeográfico, Colombia

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Durante Julio y Agosto de 2011 se efectuaron colectas a nivel de sotobosque en doce localidades en la Serranía de los Paraguas en una cota de 1500 a 2400 msnm. Cuatro de estas localidades están ubicadas en el departamento de Chocó y ocho en el departamento del Valle del Cauca, el portón al Chocó Biogeográfico. El objetivo de la expedición a la Serranía de los Paraguas en la Cordillera Occidental Colombiana, fue responder a la necesidad de análisis de endemismos de la familia Orchidaceae señalado por Silverstone-Sopkin & Ramos-Pérez en expediciones a la zona hacia 1986, señalando al Cerro El Torrá como una unidad fitogeográfica aparte de los andes colombianos. Se herborizo, fotografió y preservó en etanol 70% y glicerina 10% cada flor. Hasta el momento los resultados

arrojan 37 géneros y 160 especies. De éstas toman relevancia seis especies (3,75%) endémicas del Cerro El Torrá, ocho especies (5,00%) nuevas confirmadas o en proceso de descripción, sumadas a cinco especies (3,12%) endémicas de la Serranía de los Paraguas. Aún faltan en el análisis 54 especies (33,75%) en proceso de identificación, especialmente de los géneros *Pleurothallis*, *Lepanthes*, *Stelis* y *Maxillaria*. Ambos exploradores afirman que el número de especies en la Serranía es mayor. Son necesarias nuevas expediciones con acceso al dosel, en otras temporadas del año y en nuevas localidades para cuantificar la composición de la familia Orchidaceae en la región. Estudios como este, contribuyen al fortalecimiento de los procesos de conservación en la región.

## Taxonomy, molecular phylogenetics, reproductive isolation, and niche differentiation of the *Specklinia endotrachys* species complex (Orchidaceae: Pleurothallidinae)

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We combined a taxonomic study with molecular phylogenetics of nrITS and *trnK-matK* sequences to define species boundaries in the *Specklinia endotrachys* complex. By applying ecological niche modeling we intend to determine whether the lineages present in this group (1) inhabit different habitats and (2) are separated geographically by unsuitable habitat across a gap in the distribution. Through GC-MS analyses we are willing to investigate whether reproductive isolation also might have evolved due to switches in pollinators. Preliminary results reveal that four lineages are present in the complex. Morphological

differences include caespitose vs. repent habits, compact vs. remote inflorescences, flower color, and petal and lip shape. Molecular phylogenetic studies showed that plants with certain common morphological traits also form distinctive, well-supported clades. Preliminary niche modeling indicates that differences in suitable habitat exist among the four lineages. The lineages are separated in Costa Rica by the complex mountainous systems, with preferences for the north or south of the country, the Pacific or Caribbean watershed, and cloud or dry forests. Observations on pollinators indicate that species of the *S. endotrachys* complex are pollinated

by *Drosophila immigrans*. Further investigation will target the scent produced by the flowers and the small projections on the sepals, to which the flies are attracted. The complex investigated diverged into four allopatric

genetic lineages separated by the tall central mountain range in Costa Rica. Based on morphological and genetic differences we propose to recognize the different lineages as four separate species.

## Morphological and phylogenetic characterization of the diversity of the genus *Vanilla* in the Colombian Pacific region

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Vanilla extract, derived principally from the species *Vanilla planifolia* Andrews (Orchidaceae), is a high-value crop for cultivation in lowland, humid tropical zones, offering an important economic opportunity for the “campesino” communities in these regions. The Neotropics represent the center of diversity for the clade that produces aromatic fruits, including *V. planifolia*. Eleven species of *Vanilla* are registered for Colombia. However, this genus is poorly documented in Colombia and across the region. Although the natural distribution of *V. planifolia* is controversial, the consensus is that it is native to Central America, and plants found in other regions are the result of anthropogenic activities. The taxonomy of the genus is difficult, with many herbarium accessions being sterile and infrequent flowering in natural populations. This

study investigated the diversity of species of *Vanilla* present in the lowland humid tropical region along the Pacific coast of Colombia, combining morphological descriptors and DNA sequence data from the *matK* locus of the plastid genome and the ITS locus of the nuclear genome. Nine species were described. Three of these are new reports for Colombia: *V. bicolor*, *V. helleri*, and also a definitive identification of native *V. planifolia*. These results represent an important contribution to the knowledge of the Colombian flora, particularly of the Chocó Biodiversity Hotspot of the Colombian Pacific region. Additionally, this is an important first step in the development of sustainable vanilla production systems using native material. The promotion of effective conservation and sustainable management plans for these species is now a priority.

## Evolución de rasgos florales y vegetativos en el género *Gavilea* (Orchidaceae)

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El género *Gavilea* Poepp. (Orchidaceae) es endémico del sur de Sudamérica. En Chile habitan 11 especies del género, que se distribuyen desde la zona central (30°S) hasta el extremo sur del país (55°S),

incluyendo una especie endémica del Archipiélago de Juan Fernández. El objetivo de este trabajo fue aclarar la trayectoria evolutiva de los distintos rasgos florales y vegetativos de las especies del género

*Gavilea*. Para esto, evaluamos la señal filogenética de diferentes rasgos de estas especies, analizando cuáles caracteres son más plásticos y cuáles se han mantenido más estables a lo largo de la filogenia de este taxón. Además, quisimos evaluar si dentro de los rasgos florales había algunos que estuvieran evolucionando en forma correlacionada. Evaluamos la señal filogenética y todos presentaron un índice K de Blomberg menor a 1, no significativo ( $P > 0,05$ ) y un valor de lambda cercano a cero, sugiriendo que

los rasgos florales son evolutivamente plásticos. Sin embargo, al evaluar la correlación entre rasgos florales a través de contrastes independientes, se observó una correlación significativa entre varios de éstos, como el largo del sépalo dorsal con el largo del sépalo lateral, y el largo de las caudículas con el largo de los sépalos laterales, entre otros. Podemos concluir que los rasgos florales son evolutivamente plásticos en la filogenia de este género y que a su vez, algunos de éstos presentan una evolución correlacionada.

## Atlas of orchids of the state of Santa Catarina

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The state of Santa Catarina has a land area of 95,346,181 km<sup>2</sup>, totaling 1.19% of the total area of Brazil. The vegetation of the state consists of the following biomes: tropical Atlantic Forest coast, consisting of mangroves, dunes, beaches, and sandbanks; subtropical forest of Uruguay, with small vegetation; *Araucaria* forest, which is primarily composed of pine, cinnamon, cedar, yerba mate, and tree fern. In the plateau region we find the fields, the coldest region of the state. Despite representing only 1.19% of the total area of Brazil, the state is rich in the quantity and quality of orchids. Without doubt the most famous is *Laelia purpurata*, the official flower of the state of Santa Catarina and its capital Florianópolis. The Orchid Atlas of Santa Catarina is a unique initiative to disclose clearly and objectively the occurrence of the Orchidaceae there. The methodology for the Atlas is basically to 1) research the 14 herbaria in the state of

Santa Catarina, as well as São Paulo and Rio de Janeiro, trying to identify the vouchers from Santa Catarina; 2) review existing literature for Orchidaceae of Santa Catarina; 3) perform field work in all biomes of Santa Catarina over two years, trying to identify the species found and seeking confirmation of what has been deposited in the herbaria as well as other collections that perhaps have not yet been identified or registered for the state; 4) use GPS and GIS to prepare thematic maps of biomes and the location of the species found; and 5) identify, map, and photograph the biomes, listing for each one the genera and species occurring there as well as botanical descriptions and photos of the habitat, the plant as a whole, and the individual flower. So far we have found and identified 119 works, 465 orchid species, two subspecies, four varieties, and 16 endemic species of Santa Catarina. Monitoring of project results can be viewed at [www.orquidarionsdodesterro.com.br](http://www.orquidarionsdodesterro.com.br).

## Orchidaceae of Campeche Island, Florianópolis, Santa Catarina

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Campeche Island was declared a National Landscape and Archaeological Site through the Ministerial Decree No. 270, July 2000. It has an area of 4.0 km<sup>2</sup>. The existing plant diversity in the Island of

Campeche is provided by the different habitats found there: 1) sandy coastline, characterized by having a poor soil consisting mainly of fragments of quartz (0.2 to 10.0 mm in size), high permeability to water, and

high salt content. The plants found there are psamófito-halophytes, small in size with abundant roots and long, leathery leaves; 2) rocky coastline, characterized by the presence of *Dyckia encholirioides* (Bromeliaceae); 3) forest, with many rocky outcrops and sparse herbaceous layers because the penetration of light in most places is low. This study was conducted between 2010 and 2011, in conjunction with the graduate program at the University of Lavras-MG - Botany Ornamental Plants. Herbarium specimens deposited in the Herbaria Barbosa Rodrigues (RBR) and the Federal University of Santa Catarina (FLOR) were consulted and studied. The field work on the island of Campeche occurred over the years 2010 and 2011, totaling 49 trips lasting three days each. By the end of this work, 25 genera

and 34 species of orchids and a natural hybrid were catalogued, described, and photographed. The genera and species found were: *Acianthera pubescens*, *A. serpentula*, *A. saundersiana*, *A. sonderana*; *Aspidogyne bidentifera*; *Brassavola tuberculata*; *Campylocentrum aromaticum*; *Catasetum cernuum*; *Cattleya leopoldii*; *Cyrtopodium flavum*; *Cleistes macrantha*; *Encyclia odoratissima*; *Epidendrum fulgens*; *Gomesa crispa*; *Maxillaria picta*; *Miltonia flavescens*; *Notylia longispicata*; *Octomeria grandiflora*, *O. montana*, *O. diaphana*; *Oeceoclades maculata*; *Oncidium pumilum*, *O. flexuosum*, *O. ciliatum*, *O. barbatum*; *Ornithocephalus myrticola*; *Polystachya estrellensis*; *Prescottia densiflora*; *Rodriguezia decora*; and *Stanhopea graveolens*.

### ***Cattleya labiata* Lindl. and its varieties - a reflection**

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*Cattleya labiata* was discovered in 1818 by William Swainson during his scientific expedition to Brazil. It was described by English botanist John Lindley in 1821. The specific plant came from northeastern Brazil, far from the coast and 500 to 1000 m above sea level where the temperature oscillates between 18 and 22 C. The varieties of *Cattleya labiata* have always been subjects of much controversy among Brazilian hobbyists. A variety in the orchid sense should be based on the existence of more than one factor, including

flower shape, color, design, texture, size, and substance. Over the past 20 years several authors and Brazilian organizations, such as L. C. Menezes, João Paulo de Souza Fontes, Federation of Orquidofilia Gaucha, and Federation of Santa Catarina Orquidofilia, have created their own lists of varieties. According to these works and combining information from charts and table, 37 varieties of *Cattleya labiata* have been described based on the color and shape of the flower and 12 varieties based on the design of the lip.

### **Novelties in Orchidaceae for the Colombian flora**

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During floristic inventories conducted in remnant cloud forests and páramos from the western and eastern Cordilleras of the Andes, several new species and chorological novelties have been reported as the

result of intensive field and herbarium work since 2009. Material from each species found was collected and documented with pictures and field notes; several dried specimens from the most representative herbaria

in the country were also studied. The study and description of all new taxa were supervised by many orchid specialists. Twenty-seven new species and three new chorological records for the Colombian flora have been reported, represented as following for these genera: *Acianthera* Schweid. (2 new species), *Campylocentrum* Benth. (1 new species), *Epidendrum* L. (13 new species), *Lepanthes* Sw. (7 new species and two new records), *Lockhartia* Hook. (1 new species), *Stelis* Sw. (1 new species), and *Telipogon* Kunth (2 new species and one new record). Despite

the high level of perturbation of these ecosystems and human pressures, orchid diversity and the amount of endemism are surprisingly high compared to those of similar, protected ecosystems from the same region. Nevertheless, the scarcity of populations of some of the new species demonstrates 1) the importance and utility of floristic inventories and protection of the orchid diversity of Colombian forests and páramos and 2) the need for urgent short- and long-term conservation strategies in order to save Colombian wild orchid populations from extinction.

### **Anotaciones sobre la composición de la comunidad de orquídeas en la Reserva Natural Bosque de Yotoco (Valle del Cauca, Colombia)**

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Durante el año 2009 se estudió, en La Reserva Natural Bosque de Yotoco, la flora orquídeológica que caracteriza este bosque, uno de los últimos relictos boscosos del Valle del Cauca, Colombia. Con el fin de capturar la diversidad de esta familia de hierbas terrestres, rupícolas como epifitas; se trazaron de manera sistemática 104 transectos (69 verticales, 29 horizontales, 5 lineales) dentro del bosque, cubriendo

más de 30 000 m<sup>2</sup>. Se encontraron 91 morfoespecies, 81 determinadas hasta especie, *Stelis spathulata* y *Stelis argentata* las más abundantes, los forófitos más frecuentes Corbones (*Poulseria armata*), Caimitos (*Pauletia caimito*) y Lauraceas, sin embargo los más diversos fueron *Eugenia* sp. y *Ficus insipida*. Se registraron 19 nuevas especies, para un aproximado de 100 especies reportadas en la Reserva.

### **Checklist and illustrated guide to the Cauca River Valley's orchids and the Andean foothills (southwestern Colombia)**

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As part of the principal author's doctoral thesis, 21 dry forest patches were explored in the Cauca River Valley bioregion (421,000 ha) in southwestern Colombia.

Sampling was carried out from September 2009 to October 2010 at elevations between 930 and 1200 m. Field work was carried out during 346 hours over a

distance of 60 km. This exploration produced the most complete list of orchids ever made in this territory, with a total of 70 species belonging to 41 genera. This list exceeds the previous record by 37 species (112%) and represents 1.74% of the total of orchid species reported for Colombia. Several new regional records were found, as well as at least three endemic species and probably one local extinction. We detected five centers of high orchid concentration (“regional hot spots”), each with 21–29 species. We found that total species richness of this bioregion (70 species in 421,000 hectares) is 842 times

lower than the much smaller Yotoco forest adjacent to this area (69 species in 500 hectares). Possible explanations might be that plant diversity decreases when rainfall is low, and this is valid for orchids as well. However, other factors such as 130 years of habitat destruction (only 2% of dry forest remains) lead us to think that extinction has occurred. At the same time, an illustrated guide of these species was printed to increase public awareness of the importance of orchid conservation. Also, a digital high-resolution bank of images of the orchid flora of the department of Valle del Cauca was begun.

## The orchid flora of Chorogo Wildlife Reserve, Puerto Armuelles, Chiriquí, Panamá

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We performed a preliminary taxonomic study of the orchid flora of Chorogo Wildlife Reserve, Chiriquí, Panama. Chorogo is located 11 km west of Puerto Armuelles along the Panama-Costa Rica border and protects about 1,000 ha of lowland tropical wet forest with elevations of 344–572 m, average annual temperature of 27 C, and 226.5 mm of precipitation. Samples were collected at different sites of the reserve during three field trips. The material was cultivated and documented electronically by photographs and botanical plates with stereoscope, digital camera, and scanner. Specimens are kept in the reference collection of dried specimens, living plant collection, and spirit collection at the UCH herbarium. Identification was made by comparing the

specimens with types and protologues. The information is available free online at the website Epidendra (www.epidendra.org). Composite plates, distribution, descriptions, ecology, etymology, synonymy, taxonomy, and photographs are provided for each of the 15 species belonging to 11 genera recorded. Of these, seven are new records for the province of Chiriquí. *Epidendrum coronatum* and *Mormodes fractiflexa* are considered vulnerable by national conservation criteria. Together with studies conducted in the Pacific of Costa Rica, this project aims to strengthen the understanding of the orchid flora protected by both countries on the Pacific lowlands. It also represents the beginning of the study of the orchids of the protected areas of Panama.

## What delimitation for *Stelis* should be used?

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The use of DNA sequences for the phylogenetic reconstruction of subtribe Pleurothallidinae has led to a new delimitation of *Stelis*, and the genus now includes several groups that are morphologically

heterogeneous (*Stelis* s.l.). Some authors do not accept this broader delimitation and recognize *Stelis* in its narrow sense (*Stelis* s.s.), raising to generic level those related groups (*Crocodelanthae*, *Dracontia*,

*Effusiella*, *Elongatia*, *Lalexia*, *Mystacorchis*, *Niphanta*, *Physosiphon*, *Physothallis*, *Rhynchopera*, *Salpistele*, *Talpinaria*, and *Unciferia*). Based on analyses that have evaluated the phylogenetic relationships of *Stelis*, in this work we compare and discuss both proposals. The concept of *Stelis* s.l. encompasses a monophyletic group with variable morphology among its members but with strong support. Against this, it has been argued that there are no morphological synapomorphies that define the group and, hence, it is preferable to divide it. However, this consideration forgets the fact that morphology is not the only criterion useful in

delimiting a taxonomic group. On the other hand, following this approach leads to taxonomic inflation and complicates a classification system that otherwise would be easier to learn and use. Some groups segregated from *Stelis* s.l. are not monophyletic (*Dracontia*, *Effusiella*); others are monotypic and defined by autapomorphies (*Lalexia*, *Mystacorchis*). Furthermore, more phylogenetic work is necessary to evaluate the position of some species previously transferred to *Stelis* s.l. that seem to be more closely related to *Pleurothallis* (e.g. *Stelis quadrifida*, *S. restrepioides*).

### A newly recognized clade of *Pleurothallis* with Mesoamerican distribution

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Phylogenetic studies of *Pleurothallis sensu lato* have revealed a previously unrecognized clade of *Pleurothallis* species, including to date: *Pleurothallis aurita*, *P. bitumida*, *P. bogarinii*, *P. cobriformis*, *P. dorotheae*, *P. excavata*, *P. nitida*, *P. saccatilabia*, *P. sanchoi*, *P. scaphipetala*, *P. simulans*, and two as yet undescribed species. These species were previously considered part of *Pleurothallis* subgenus *Pleurothallis* section *Macrophyllae-Fasciculatae*, but this section was segregated from *Pleurothallis* by Luer and along with *P.* section *Pleurothallis* subsection *Acroniae* was raised to generic level as *Acronia*. In an alternate interpretation by Szlachetko *et al.*, *P.* section *Macrophyllae-Fasciculatae* was alone segregated from *Pleurothallis* as genus *Zosterophyllanthos*. The aforementioned species, with the exception of *P. bogarinii*, are listed under these alternate genera in the World Checklist of

Selected Plant Families, but the names are considered synonyms of *Pleurothallis*. Samples of these species were obtained from collections in Costa Rica, Guatemala, Mexico, Panama, and the U.S. Analyses of nuclear ITS and plastid *rpoB* and *matK* sequences for these species reveal a well-supported clade which can be interpreted as either sister to or part of the *Pleurothallis sensu stricto* clade but which is distinct both from the clade containing the other species of *P.* section *Macrophyllae-Fasciculatae* and from the clades containing species of *P.* section *Pleurothallis* subsection *Acroniae*. These phylogenetic data, differences in floral and foliar morphology from other species in *P.* section *Macrophyllae-Fasciculatae*; and, as far as we know, an exclusively Mesoamerican distribution (Costa Rica, Guatemala, Mexico, and Panama), strongly indicate that a new subgenus of *Pleurothallis* is warranted for the clade.



## A preliminary phylogenetic analysis of *Pleurothallis sensu lato* based upon nuclear and plastid sequences

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Morphological studies of *Pleurothallis sensu lato* by Luer and by Szlachetko have led them to propose several segregate genera, including *Acronia*, *Ancipitia*, *Colombiana*, *Elongatia*, *Lindleyalis*, *Lalexia* (for *P. quadrifida*), *Mirandopsis*, *Rhynchopera*, *Talpinaria*, and *Zosterophyllanthos*, leaving a much smaller group of species in *Pleurothallis sensu stricto*. In contrast, in molecular phylogenetic analyses by Pridgeon *et al.*, species in the proposed taxa *Ancipitia*, *Colombiana*, *Mirandopsis*, *Pleurothallis*, *Lindleyalis*, *Rhynchopera*, and *Talpinaria* formed a clade, indicating *Pleurothallis* should be defined more broadly; however, the analyses included only 15 species in total from these groups. The current investigation includes several species from each of the proposed segregate genera (excluding *Mirandopsis*) as well as from each of the groups within *Pleurothallis sensu stricto* (*Antenniferae*, *Longiracemosae*, and *Macrophyllae-Racemosae*),

for a total of ~143 ingroup species. Nuclear ITS and plastid *matK* markers have been sequenced for the majority of the species, and sequencing of 3' *ycf1* and *trnL-F* is underway. Preliminary analyses indicate that the species sampled from the taxa *Acronia*, *Ancipitia*, *Colombiana*, *Lindleyalis*, *Lalexia*, *Pleurothallis*, *Rhynchopera*, and *Talpinaria* do form a well-supported clade, as anticipated from the results of Pridgeon *et al.* However, from the proposed genus *Elongatia*, only the species *Elongatia excelsa*, *E. macrophylla*, *E. restrepioides*, and *E. sijmii* are included in the clade. Internally, there are well-supported distinct clades for species in the groups *Ancipitia/Colombiana*, *Lalexia*, *Lindleyalis*, *Loddigesia*, *P.* section *Macrophyllae-Fasciculatae* (excluding Mesoamerican species in the *P. excavata*-group), and *Rhynchopera*. *Pleurothallis* section *Pleurothallis* subsection *Acroniae* is not monophyletic, its members distributed among three clades.

## ECOLOGY

## Patrones de distribución vertical y horizontal de la epífita endémica *Prosthechea* aff. *karwinskii* (Orchidaceae) en Michoacán, México

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*Prosthechea* aff. *karwinskii*, orquídea epífita endémica de Michoacán, México, con solo una población conocida. El presente estudio tiene como objetivo determinar los patrones de distribución vertical y horizontal de *P.* aff. *karwinskii* de acuerdo a su estructura poblacional. Se censaron 355 orquídeas considerando: largo y ancho del pseudobulbo más joven bien desarrollado, número de eventos reproductivos, sección y zona del forofito, orientación, posición en la rama y sustrato. Los análisis estadísticos se realizaron a cada clase de vida (plántula, juvenil, adulta 1 y adulta 2), contemplando un P-valor < 0.05. Todas las clases de vida presentaron mayores frecuencias en la sección copa, en las zonas del forofito las plántulas se distribuyeron homogéneamente y las demás clases

registraron mayores frecuencias en la parte media y parte interna de las ramas, las orientaciones Este, Sur y Oeste, resultaron las más frecuentes; en la posición en la rama las plántulas tuvieron mayor frecuencia en la parte lateral y en ramas verticales y las adultas 2 en la posición lateral y abajo; las plántulas presentaron la mayor frecuencia sobre sustrato de líquen, las juveniles sobre líquen y corteza y las adultas tuvieron mayor afinidad por la corteza. La distribución resultó más homogénea en las primeras clases de vida y con tendencia a ciertas variables conforme avanza el ciclo de vida, también se observó una diferencia entre los sitios donde la semilla germina y aquellos donde las plantas finalmente se establecen. Se observó que *P.* aff. *karwinskii* tiene como hospedero específico a *Quercus deserticola*.

## Horizontal and vertical distributional patterns of epiphytic Orchidaceae in a cloud forest in Colombia

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Utilizing canopy ascent techniques in a cloud forest in the Western Cordillera of the Andes in Colombia, 25 trees with diameter at breast height (DBH)  $\geq$  30 cm were climbed. In each tree, the location of epiphytic orchids was noted to determine location preferences in certain zones on the trees and whether the orchids prefer trees of a certain size distributed in three diameter categories: small trees (30-35 cm DBH), medium-sized (35-50 cm DBH), and large (DBH > 50 cm). A total of

1,813 individuals of 44 species of orchids belonging to 14 genera was registered. Analysis of variance carried out for 23 species represented by more than five individuals and located in at least three trees revealed that 12 species showed a tendency to be located on trees of a certain size, in different elevational zones on trees, or both. *Elleanthus* sp.1, *Maxillaria aurea*, and *Stelis* sp.1 showed significant differences for both of these parameters. Two other analyses were carried out, one

for total abundance and another for species richness, to determine if there were differences between different categories of diameter and different elevational zones within trees. Results showed greater abundance and richness of orchids in the canopy of the medium-sized

trees. Thus, estimates of plant diversity in cloud forests including the canopy are important. With regard to size of trees, it could be argued that the three diameter categories are associated with different temporal stages in the population dynamics of these orchids.

## Reproductive biology of *Masdevallia coccinea* and *Masdevallia ignea* in Guasca (Cundinamarca: Colombia)

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*Masdevallia coccinea* and *Masdevallia ignea* (popularly known as “banderitas”) are ornamental orchids which are prized by amateur farmers and collectors. In Colombia, the harvest pressure on these species has been enormous, and few natural populations survive. Therefore, both species are on Appendix II of CITES. The goal of the project was to study the reproductive biology and pollination biology of *M. coccinea* and the *M. ignea* (Pleurothallidinae) under semi-cultivation conditions in the Villa Rosa farm located in the Municipality of Guasca, Cundinamarca (Colombia). For both species we studied floral morphology, phenology, breeding system, floral visitors, and pollinators, as well as visual and

chemical cues. *Masdevallia coccinea* and *M. ignea* differ in floral color and in length of the sepals. In both species the labellum is articulated at the base of the column, but the lip is devoid of nectar and osmophores. Both species flower asynchronously and are self-compatible but pollinator-dependent. *Masdevallia coccinea* and *M. ignea* were visited by 15 and 7 insect morphospecies, respectively. Both orchid species were pollinated by female flies (Diptera: Drosophilidae) that performed different activities on the flowers. The flowers of *M. coccinea* and *M. ignea* reflected through all wavelengths such as UV, blue, and red. Floral volatiles of *M. coccinea* and *M. ignea* were also different.

## Identificación de bacterias endófitas asociadas a raíces de *Cattleya quadricolor* Lindl.

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Las bacterias endófitas se encuentran colonizando los tejidos internos de las plantas sin ocasionar infección y aportando características competitivas importantes para éstas. El objetivo de este trabajo fue identificar especies endófitas asociadas a raíces de *C.*

*quadricolor*. Los aislamientos se realizaron lavando las muestras con agua corriente, desinfectando con hipoclorito de sodio 1%/3min, etanol 50%/3min y agua destilada estéril; posteriormente, se retiró la corteza y se maceró el cilindro central, sembrando

0.1mL del macerado sobre agar Nutritivo y agar Infusión Cerebro Corazón, y extendiendo con asa de Digralsky; las cajas fueron incubadas a 28°C hasta observar desarrollo de colonias, las cuales fueron caracterizadas por marcador morfológico macroscópico y microscópico mediante tinciones de Gram para la determinación de reacción, forma y disposición. De las raíces procesadas se obtuvieron dos morfotipos; el morfotipo CQB1 de colonias circulares, borde entero y continuo, tamaño promedio de 2mm, superficie lisa y conformado por bacilos Gram-positivos sin disposición celular. El morfotipo

CQB2, de colonias grandes (5mm), irregulares, convexas bajas, superficie rugosa, consistencia cremosa, borde ondulado y conformado por bacilos Gram-positivos sin disposición celular. A la fecha, se han obtenido dos morfotipos bacterianos los cuales serán identificados molecularmente a nivel de especie y evaluados para determinar su actividad biológica sobre *C. quadricolor*, subrayando el papel importante que juegan los microorganismos en los ecosistemas y teniendo en cuenta el escaso conocimiento que existe de estas bacterias y su función en las raíces de las plantas.

### Mycorrhizal and endophytic fungal communities associated with roots of *Pseudorchis albida* (Orchidaceae)

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Orchid mycorrhizal symbiosis constitutes one of the most significant specializations in orchid evolution. Most importantly, orchid mycorrhizal fungi (OrMF) are essential for orchid seed germination and further development and determine the carbon nutrition of non-photosynthetic species. Together with OrMF, orchid roots harbor a miscellaneous group of non-mycorrhizal root associated fungi (RAF), which belong to the omnipresent group of plant fungal endophytes. Despite their ubiquity and cosmopolitan distribution, orchid RAF have been rarely studied. Methods used for assessing OrMF and RAF diversity in plant roots recently shifted from culture-dependent to culture-independent approaches. In the present study, the endangered photosynthetic orchid *Pseudorchis albida* was screened for OrMF and RAF presence using culture-dependent and culture-independent

techniques. The efficiency of the three different approaches was evaluated as well as the effect of sampling season. Sixty-six distinct OTUs of mycorrhizal and non-mycorrhizal fungi were found in *P. albida* roots in total. The OrMF community was dominated by *Tulasnella* species, which were mainly detected by isolation from pelotons or the culture-independent technique (direct DNA isolation from roots and cloning). The vast majority of RAF species belonged to the order Helotiales and had surprisingly wide putative ecological amplitude. Although the diversity and community assemblages of RAF were higher using the culture-independent technique, three of the seven most abundant RAF were exclusively detected by the culture-dependent approach. A combination of both methodological approaches seems to be the best way to study RAF diversity in orchid roots.

## The genus *Neottia*, an early step in shift from autotrophy to mycoheterotrophy in orchids?

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Many plant families evolved mycoheterotrophy as a way of gaining nutrients, but the evolution of such nutritional shift has been little studied. We investigated mycorrhizal associations and a nutritional mode of two orchid species, a common ecological generalist *Neottia ovata* and *N. cordata*, a species of peatbog spruce forests. The species are closely related to a non-green *N. nidus-avis*, associated with ectomycorrhizal Sebaciales, clade A. We investigated the course of germination at 18 sites in the Czech Republic, analyzed fungal spectra of both seedlings and 39 *N. ovata* and 21 *N. cordata* adults growing in 32 European sites in different habitats and measured the natural content of stable isotopes. Seeds of both species suffered high mortality during two years of *in situ* germination. Whereas *N. ovata* produced few protocorms at both

*N. ovata* and *N. cordata* localities, *N. cordata* never reached a protocorm stage. We found Sebaciales, clade B, in both seedlings and adults, independent of habitat type and site geography. The spectra of accompanying fungal species differed according to habitat: the meadow individuals associated frequently with saprophytic Tulasnellaceae, whereas the forest individuals associated more often with ectomycorrhizal fungi. Natural content of stable <sup>13</sup>C and <sup>15</sup>N isotopes in forest specimens, however, did not indicate significant nutrient gain by mycoheterotrophy. *Neottia* species form mycorrhiza with Sebaciales, clade B, in various habitats. This association together with the co-occurrence of ectomycorrhizal fungi at forest sites could serve as a predisposition for the shift to full mycoheterotrophy in *Neottia*.

## Endophytic fungi associated with natural populations of *Vanilla* species in south-west Colombia

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The genus *Vanilla* (Orchidaceae) is of economic interest as the source of the natural vanilla fragrance, derived principally from cultivated plants of the species *V. planifolia*. The clade of fragrant species (*V.* subgenus *Xanata*, section *Xanata*) has a natural distribution in the Neotropics. Work in this research group has identified populations of ten *Vanilla* species in the region. Orchid species, including those of *Vanilla*, have an intimate relationship with mycorrhizal fungi to provide nutrients during seedling establishment and often in mature plants. Colombia has over 3,300 orchid species, but

little is known regarding the diversity of orchid mycorrhizal fungi essential for the establishment and survival of these species. We aim to characterize the diversity of fungi associated with the root system of adult plants of *Vanilla* species native to the diverse ecosystems of south-west Colombia, which include both humid tropical rainforest and sub-xerophytic habitats. Three methodologies are being implemented: 1) isolation of fungi from root portions colonized with mycorrhizal fungi; 2) isolation from single pelotons; and 3) identification of fungi present through direct PCR amplification using

fungal specific PCR primers. All fungi are identified by DNA sequencing of the ITS locus. Additionally, fungi are evaluated for their mycorrhizal potential through assays of symbiotic germination of *Vanilla* seeds. The results support the bioprospecting focused on the con-

servation of these valuable plant genetic resources, as well as the development of sustainable cultivation techniques. This study is also an important contribution to the incipient studies of microbial diversity in the megabiodiverse country of Colombia.

## Hongos micorrízicos de orquídeas en poblaciones naturales en los departamentos del Valle del Cauca y Antioquia, Colombia

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En Colombia existe una amplia diversidad de especies de orquídeas (cerca de 3.500 especies), muchas aún no descritas. También, dentro de la diversidad se incluyen los hongos micorrízicos del género-forma Rhizoctonia y sus teleomorfos en los géneros *Thanatephorus*, *Sebacina*, *Ceratobasidium* y *Tulasnella*. En algunas orquídeas terrestres de zonas templadas se asocian con otros grupos de hongos. Para esta zona tropical es poco lo que se conoce sobre sus hongos micorrízicos y aún no es claro si la mayoría de orquídeas, se asocian con los mismos micosimbiontes o si existen otros cumpliendo tal actividad. Para contestar esta pregunta se tomaron secciones de raíces micorrizadas, de las cuales se

aislaron y cultivaron hongos para secuenciar ADN de la región ITS nuclear ribosomal. Igualmente se realizaron ensayos de germinación simbiótica. Estudios desarrollados por el Grupo mostraron a *Ceratobasidium* como el género de hongo micorrízico predominante en algunas orquídeas de Colombia con hábito terrestre, epífita y hemiepífita (*Vanilla* spp., en raíces terrestres). Las secuencias de hongos de orquídeas epífitas se relacionaron estrechamente con secuencias de hongos de orquídeas epífitas de Puerto Rico. Lo anterior sugiere que *Ceratobasidium* está presente como micorrízico en orquídeas independiente del hábitat de la planta y su ubicación geográfica.

## Selección de rasgos florales en *Rodriguezia granadensis* (Lindl.) Rchb.f. (Orchidaceae): estudio de la eficacia biológica en una especie polimórfica

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La supervivencia de las especies depende en parte de su dinámica poblacional y sus características fenotípicas. El proceso de selección natural es un mecanismo que puede influenciar la producción de

progenies. Se estudió la biología y demografía de dos poblaciones de *Rodriguezia granadensis* (Lindl.) Rchb. f. en la zona de la Reserva Forestal Bosque de Yotoco y en el corregimiento El Dorado, Colombia,

para determinar si hay variación en la adecuación Darwiniana de dos poblaciones. La población de Yotoco presenta dos fenotipos claramente diferenciables por el color (Rosa y Blanco), mientras que en Centenario se encontró solamente el fenotipo Blanco; las flores pueden presentar o no néctar independiente de su fenotipo. Las ofertas florales de *R. granadensis* se encuentran en el callo del labelo y el nectario de ducto que contiene el espolón o sinsépalo, recubierto por un epitelio glandular compuesto por osmóforos y nectarios. *R. granadensis* es polinizada por *Eulaema* sp. que la visita en

busca de alimento, es autoincompatible, xenogama obligada y dependiente del polinizador. Mediante simulaciones con matrices demográficas de transición a 50 años y 500 repeticiones se encontró que ambas poblaciones y fenotipos tienden a la extinción en un plazo de 25 años. No se encontraron diferencias entre la adecuación Darwiniana, de los fenotipos de estudio por lo que no se puede probar la existencia de selección fenotípica sobre los caracteres florales. La variación de la especie puede deberse a la deriva génica o la selección dependiente de la frecuencia y la ecología cognitiva del polinizador.

### Distribution patterns of *Cuitlauzina pendula* La Llave & Lex (Orchidaceae) over its phorophytes at the ‘Barranca de Cupatitzio’ National Park, in Uruapan, Michoacán, México

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*Cuitlauzina pendula* La Llave & Lex. is an epiphytic, endemic, and threatened Mexican orchid. This research was carried out at the ‘Barranca de Cupatitzio’ National Park in Uruapan, Michoacán. The objectives of this study were to 1) evaluate vertical and horizontal distribution of *C. pendula* and 2) determine if there are preferences, taking into account the age class in the position on the tree (side, up, and down), substrate type (bark, moss, and lichen), as well as the orientation (N, S, E, W). Two quadrants of 12.5 x 25 m were established in homogeneous conditions of oak-pine forest, and two more quadrants of the same size at a pine-oak forest. Records were made for the host type, number of individuals in the different zones of the phorophytes (tree stem, tree canopy, and the respective sections

of the tree canopy), age classes (seedlings, juveniles, and adults), position on the branches, substrate type, and orientation. Results of this study show greater abundance of *C. pendula* on the tree canopy (91.6%) and on the middle part of the branches (43.9%). The population is mainly represented by the adult class (62.3%) with more individuals on the lateral position (54.5%) and on the bark-moss substratum (76.7%), the east (19%), and north position (16.5%). *Cuitlauzina pendula* has a significant greater number of individuals in the oak-pine forest than in the pine-oak forest. In light of these results, this study could be employed as a tool to generate appropriate strategies of management that promote the conservation of this species.

## Mating system and reproductive success of *Cuitlauzina pendula* La Llave & Lex (Orchidaceae) at the National Park 'Barranca del Cupatitzio' of Uruapan, Michoacán, México

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Studying reproductive biology is useful to promote conservation of species. *Cuitlauzina pendula* La Llave & Lex. is an epiphytic, threatened species. The objectives of this work were to 1) determine if the species requires the visit of pollinators to produce fruits and seeds and 2) evaluate the mating system and reproductive success in terms of fruit production and viability of the seeds. For the evaluation of the mating system, 20 inflorescences were randomly selected, and the following manual pollination treatments were applied: self-pollination, cross-pollination, natural pollination. Another 20 more inflorescences were left uncovered and unmanipulated in order to evaluate natural pollination. Developed fruits were supervised until ripeness. From each of the obtained capsules, the viability of 100 seeds was evaluated with

an optical microscope on the basis of the presence and development of embryos, assigning to the seeds the following categories: a: empty; b: developing embryo 1; c: developing embryo 2. Results show low fruit set values for all treatments (5% self-pollination, 7.5% cross-pollination, and 3.16% natural pollination). On the subject of viability of the seeds, treatments showed significant differences with regard to the development categories ( $\chi^2=247.035$ ,  $gl=4$ ,  $P=0.000$ ). *Cuitlauzina pendula* is a preferably exogamous species that requires pollinators to achieve sexual reproduction, and taking into account that the number of fruits produced with the outcrossing pollination treatment is greater than with the natural pollination treatment, pollination limitation of the species could be considered.

## Identificación de bacterias endófitas asociadas a raíces de *Vanilla* spp. (Orchidaceae)

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Las bacterias endófitas promueven el crecimiento vegetal, controlan fitopatógenos y participan en la asimilación de nitrógeno. El objetivo de este trabajo fue caracterizar bacterias endófitas de raíces de *Vanilla* spp. nativas al Valle del Cauca, Colombia. Las muestras fueron lavadas con agua corriente, se desinfectaron con hipoclorito de sodio 1%/3 min., etanol 50%/3 min., lavadas con agua destilada estéril, se les eliminó la corteza y el cilindro central fue macerado y sembrado en caldo Infusión Cerebro Corazón; se incubó a

28°C/4d. Del caldo se extrajeron diariamente 10µL y se estriaron sobre la superficie de agar Nutritivo, el cual se incubó a 28°C hasta la observación de colonias, las cuales fueron caracterizadas por marcador morfológico macroscópico y microscópicamente mediante tinciones de Gram para la determinación de forma y disposición. Concluida la incubación, se observaron dos morfotipos de colonias bacterianas: el morfotipo VP1 de colonias circulares beige mayores a 1mm de diámetro, elevación convexa baja, borde entero, superficie lisa, consistencia



mucoide y aspecto brillante y, conformado por bacterias bacilares Gram-positivas sin disposición celular; y el morfotipo VP2, de colonias circulares beige mayores a 1mm de diámetro, elevación convexa baja, borde entero, superficie lisa, consistencia cremosa y aspecto brillante y, conformado por bacterias en forma de

coco, Gram-positivas sin disposición celular. Estos dos morfotipos bacterianos serán identificados a nivel de especie y actividad biológica en *V. planifolia*. Este trabajo representa un aporte importante al conocimiento de la comunidad microbiana asociada a las especies de *Vanilla* nativas de Colombia.

## ***Ceratobasidium* lineages associated with two epiphytic orchids in Colombia**

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Extremely small orchid seeds lack endosperm, which is indispensable for seed germination. In nature the association with suitable mycorrhizal fungi provides simple sugars during the first steps of germination. Colombia is one of the countries with the greatest biodiversity of orchids, with an estimate of 3,200 species, but few studies on orchid mycorrhiza have been performed. In our study we isolated and sequenced the ITS rDNA region of fungi from two sympatric, epiphytic orchids of Colombia, *Ionopsis utricularioides* and *Psycmorchis pusilla*, both belonging to subtribe Oncidiinae. All sequences were recognized as belonging to the genus

*Ceratobasidium*, known to be a common orchid mycorrhizal fungus in both tropical and temperate orchids. One sequence was 100% similar to fungi isolated from *I. utricularioides* in Costa Rica in a previous study. *Ionopsis utricularioides* was confirmed to be a specialist, associating with only one clade of mycorrhizal fungi, whereas *Psycmorchis pusilla* proved to be a generalist, associating with three clades. This finding confirms that variation in mycorrhizal specificity is an important factor in co-existence of orchids. The high affinity between the subtribe Oncidiinae and *Ceratobasidium* was also confirmed.

## CONSERVATION SCIENCE

### Un método sencillo para sembrar orquídeas de semillas sin condiciones asépticas

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La siembra de orquídeas a partir de semillas generalmente requiere medios de cultivo y ambiente asépticos. Este requisito limita su práctica a laboratorios equipados con autoclaves y cámaras de flujo laminares, y excluye mucha gente interesada en cultivar orquídeas de semillas para desarrollar nuevos híbridos y para propósitos de conservación. Aquí se describe un método sencillo para formular gránulos o pelotillas de alginato que contienen semillas de orquídeas, un hongo micorrízico y nutrientes. El método no requiere condiciones asépticas ni equipos de laboratorio, y los ingredientes son de precio módico.

Funciona muy bien con semillas de híbridos comerciales de *Epidendrum* y *Dendrobium*, y con varias especies de orquídeas epífitas. No ha funcionado con unas de las especies probadas, quizás por que no se han identificado los hongos micorrízicos apropiados. No obstante, un hongo micorrízico del género *Ceratobasidium* ha funcionado con una variedad de orquídeas epífitas. Se espera que este método nuevo facilite el cultivo de orquídeas a partir de semillas para fines de conservación y horticultura. También se espera que el método estimule investigación sobre relaciones micorrízicas de orquídeas.

### Influencias de tres niveles de agua de coco en la germinación *in vitro* de *Rhynchostele biconiensis* (Bateman) Soto Arenas & Salazar, en medio de cultivo Knudson C

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*Rhynchostele biconiensis* (Bateman) Soto Arenas & Salazar, es una orquídea nativa de la región Soconusco, Chiapas, con distribución entre México y Centroamérica. Con el fin de establecer un protocolo eficiente de germinación asimbiótica para su conservación, se midió el desarrollo de las semillas germinadas en medio de cultivo Knudson C (1946), comparando entre 4 niveles diferentes de agua de coco añadida al medio (Control: 0 mL/L, Trat1: 75 mL/L, Trat2: 150 mL/L, Trat3: 260 mL/L). Se analizaron las variables: G(%): porcentaje de germinabilidad a 150 días de la siembra; T1: número de días para observar el primer protocormo en germinación; T50: tiempo para alcanzar el 50% de germinación; Mdays: tiempo promedio de germinación; Tmax: tiempo para alcanzar el máximo de la germinación; MR: tasa de

germinación; CVt: coeficiente de variación de Mdays; Z: índice de sincronía y U: incertidumbre asociada a la distribución de la frecuencia relativa de germinación. Para aspectos cualitativos, se consideraron también clorosis y oxidación. Los resultados evidenciaron que el agua de coco mejora la germinación, en específico la variable G, evidenció diferencias estadísticas significativas ( $p=0.002$ ) con un valor de 97% en Trat3 contra 83% del control. También, Mdays mostró diferencias estadísticas significativas ( $p=0.002$ , 134 días por el Trat3 contra 124 días del Control) así como MR ( $p=0.002$ , 0.0075 del Trat3 contra 0.0079 del Control). La sincronía del evento germinativo Z fue diferente de manera significativa ( $p=0.045$ ), así como el ln de U ( $p=0.015$ ). Los eventos clorótico y oxidativo se manifestaron solamente en el Control y Trat1.

## Research Group in Orchids, Ecology and Plant Systematics: an inter-institutional initiative for plant conservation and sustainable use in south-west Colombia

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The Research Group in Orchids, Ecology and Plant Systematics is an alliance of researchers from several institutions in south-west Colombia dedicated to the research and sustainable management of the flora and associated ecosystems in this biodiverse region. Research activities of the group are carried out in both the biodiversity hotspots of the regions: The Chocó biogeographic region on the Colombian Pacific coast; and the Tropical Andes region. The research group was registered in the Colombian System for Science and Technology in COLCIENCIAS in 2006 and has the endorsement of the Universidad Nacional de Colombia, the Pontificia Universidad Javeriana-Cali, and the Universidad del Pacifico de Buenaventura, Colombia. That of other institutions is in process. The research lines and coordinators are as follows; however, many research projects are collaborative activities among

the different members of the group: 1. Plant Ecology (Tupac Otero); 2. Conservation Genetics & Molecular Ecology (Nicola Flanagan); 3. Diversity, Ecology and Sustainable Use of *Vanilla* (Robertulio Gonzalez); 4. Plant-Microorganism Interactions (Ana T. Mosquera-Espinosa); 5. Plant Systematics and DNA Barcoding (Philip A. Silverstone-Sopkin); 6. Pollination Biology (Marcela Cuartas-Domínguez); 7. Plant Biogeography (Guillermo Reina); 8. Orchid Conservation (Nhora Helena Ospina-Calderón). Each research line has a portfolio of projects, both completed and ongoing. Funding has been obtained at the regional, national, and international levels. Further collaborators are always welcome in order to advance knowledge of this biodiverse region and to develop successful strategies for the conservation and sustainable use of this biodiversity.

## Orchids of the cloud forests of Dapa (Valle del Cauca, Colombia): research and conservation of forest treasures

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In the framework of the project “Community building for the conservation of wild orchids and annotated list of Orchidaceae in the cloud forests

of Dapa,” funded by San Diego County Orchid Society (EE.UU), several orchid inventories were conducted in remnant cloud forests from Dapa

vicinity (Valle del Cauca, Colombia). Field and herbarium work were carried out, and material from each species found was collected and documented with pictures and field notes; several specimens from VALLE and CUVC herbaria were also studied. As a main result, 121 species of 53 genera were reported, 10 of which are new species and 2 unrecorded species for the Colombian flora. New species and records are represented as following for these genera: *Campylocentrum* Benth. (1 new species) *Epidendrum* L. (5 new species), *Lepanthes* Sw. (3 new species and a new record), *Stelis* Sw. (1 new species), and *Telipogon* Kunth (1 new record). Many educational workshops were conducted

with several community members in order to communicate to them the importance of orchid diversity and conservation of cloud forest. Cloud forests of Dapa vicinity are highly diverse and host an important number of Colombian endemic orchid species, although they were disturbed in the last 30 years by the surrounding community that selectively extracted timber trees and orchids. Primary conservation strategies concerning protection of wild orchid populations and their habitats should be formulated and executed in concert with local community and environmental authorities in order to stop the habitat loss of wild orchid populations.

## Distribution pattern, conservation status, and traditional therapeutic uses of orchids with particular reference to Solan district, Himachal Pradesh, India

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The state of Himachal Pradesh in India situated between 30°22'40" to 33°12'40" North latitude and 75°45'55" to 79°04'20" East longitude, with an altitudinal range of 350 m to 6,975m, forms the part of Trans and North Western Himalayan biogeographic provinces. Presently, extensive field surveys were made in the Solan district in the state of Himachal Pradesh during which frequent visits were made in and around the orchid-rich grasslands and forest belts. During the survey, local people and community were also interviewed to gather information on traditional therapeutic uses of these orchids, and a simple questionnaire was prepared for this purpose. The present communication provides brief notes on the distribution pattern, flowering period, conservation status, and traditional therapeutic uses of some of the

orchids from the district. However, the great orchid diversity of Himachal Pradesh is progressively declining for a number of reasons, including habitat destruction and illegal and unregulated commercial collections for ornamental and/or medicinal purposes. These factors have detrimentally affected the size and frequency of natural populations of these orchid species, and the existence of many others is threatened. If the depletion of orchids continues unchecked, a large number of species may vanish even before their existence and biological/economic importance is established. Hence, there is an urgent need to identify and document orchids from the region and find appropriate conservation strategies to protect these from becoming extinct.

## Orchid legislation in Santa Catarina, Brasil

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The state of Santa Catarina, with an area of 95,346,181 km<sup>2</sup>, represents only 1.19% of Brazilian territory. It has lush vegetation, formed by different biomes. In light of these biomes, Florianópolis -- the capital of the state and location of the island of Santa Catarina -- has always been regarded as a natural nursery. For the past 80 years this feature has made the state and its orchids coveted by individuals and companies, mainly from Europe and the USA (as well as Brazil itself), in search of wealth in the quantity and quality of our orchids. To prevent this, the state, the city of Florianópolis, and some other cities of Santa Catarina have passed a set of laws seeking the preservation, maintenance, knowledge, and environmental education for the Orchidaceae of Santa Catarina, described as follows:

- Law No. 203/1954 - Regulates trade in orchids in Florianópolis
- Law No. 1480/1976 - Declares Public Utility Orquidófila Society of Santa Catarina

- Law No. 6.255/1983 - Declares *Laelia purpurata* the flower of Catarina State
- Law No. 13.054/2004 - Declares Public Utility Federation Orquidofilia Santa Catarina
- Law No. 7.073/2006 - Declares *Laelia purpurata* the flower of Florianópolis
- Law No. 8.228/2010 - Provides for the creation of the Orchid City of Florianópolis
- Law No. 15.177/2010 - Establishes Orchid Day in the State of Santa Catarina, to be celebrated June 22, the birthday of botanist João Barbosa Rodrigues
- Law No. 8479/2010 - Establishes Orchid Day in the Municipality of Florianópolis, to be celebrated June 22, the birthday of botanist João Barbosa Rodrigues

These laws, available at [www.orquidarionsdodesterro.com.br](http://www.orquidarionsdodesterro.com.br), may serve as positive examples for environmental groups, orchid circles, and local governments.

## Orchidaceae) en Bogotá D. C. y su área de influencia

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*Masdevallia caudata* Lindl. is an epiphytic orchid with distribution in Colombia. This species has ornamental potential but is threatened and has not been studied to substantiate its use and conservation. During our research we visited ten communities and found eight populations in three communities. Horizontal and vertical distribution and its phenological state were studied and a distribution map made. Dried specimens were deposited in the Herbarium of the Jardín Botánico José Celestino Mutis (JB-JCM). Wild individuals were collected in order to study adaptation, maintenance protocol, observations of *ex situ* breeding phenology

(biology and floral formula, longevity, and artificial pollination), and asymbiotic propagation *in vitro* in the Botanical Garden José Celestino Mutis (JB-JCM). The population analyzed *ex situ* consisted of 28 individuals. Reproductive phenology was observed and documented over a period of eight months. The flowering period was around 24 weeks with flowering peaks in weeks 11 and 26. In addition, flowers that were pollinated yielded a viable fruit set in a 26.31%, the most effective from geitonogamy and xenogamy. Fruit ripening occurred over approximately 151 days. Asymbiotic propagation of seeds obtained by different methods of natural or

artificial pollination was carried out. Seeds optimally germinated on Murashige and Skoog medium to 50% with activated charcoal (AC) and Knudson C with AC and 1 mg/L of NAA, GA3, and kinetin.

## Orquideas de los bosques de niebla del suroccidente de Colombia y oportunidades para su conservación

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Basado en inventarios realizados en bosques de niebla del suroccidente colombiano, en los departamentos de Nariño, Valle del Cauca y Antioquia, se se reporta un total de 420 especies de 85 géneros; una concentración marcada de especies endémicas que representa un 30% del número total de especies registrado, diez y siete nuevos reportes para Colombia, y veinte especies nuevas para la ciencia de estos bosques en años recientes. También hay 25 especies amenazadas de las mencionadas en el Libro Rojo de orquídeas de Colombia. Para la conservación de estas orquídeas se han establecido recientemente reservas naturales privadas como Morobia, municipio de Dagua, Peñas Blancas, municipio de Cali y La Irlanda, municipio de Jamundí en el Departamento de Valle del Cauca. En el departamento de Antioquia se estableció la reserva La Mesenia en el municipio de Jardín. La reserva natural privada La Planada en el municipio de Ricaurte, Departamento de Nariño se convirtió en reserva de la comunidad indígena Awa administrada por el Resguardo CAMAWARI. Las reservas naturales mencionadas integran sus esfuerzos de conservación de orquídeas con los de manejo de cuencas hidrográficas, ecoturismo, servicios ambientales y adaptación al cambio climático.

## Medicinal orchids of India and conservation measures for their sustainable management

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Orchids are the most pampered plants for their aesthetically beautiful flowers. and these constitute one of the highly evolved families of economically important plants, the Orchidaceae. Being rich in alkaloids, flavonoids, glycosides, and other phytochemicals, these plants have been used in the local system of medicine in different parts of the world since time immemorial. Numerical strength of orchid species in India has been catalogued as nearly 1,200 species; these are variously adapted to terrestrial, epiphytic, achlorophyllous, and mixed habit (as terrestrial and lithophytes) mode. In fact, Indian orchids have been used extensively in various indigenous systems of medicine since the Vedic period, and in the Vedic scriptures there is a mention of these plants under the name *Vanda*. Some of the orchid herbs including *Malaxis acuminata*, *M. muscifera*, *Habenaria edgeworthii*, and *H. intermedia* are highly valued in Ayurvedic system of medicine. The orchids have been reported to cure many ailments including cardiac (*Eulophia dabia*), dysentery (*Satyrium nepalense*), malignancy (*Vanda testacea*), nervous (*Dendrobium nobile*), orthopedic (*Dactylorhiza hatagirea*), respiratory (*Dactylorhiza hatagirea*), and rheumatism (*Rhynchosytilis retusa*) disorders. A large proportion of orchid habitats have lost their character due to expanded agricultural and other developmental activities. Further, unregulated commercial collections have also affected the size and frequency of natural populations of several medicinally important orchid species. Though extensive research work is pursued on medicinal plants, in general, orchids

have remained almost neglected in this direction. The present communication provides an account of some medicinally important orchids from India with their biological status and suggests both *in situ* and *ex situ* conservation measures for their sustainable management in the country.

## Plan de manejo para la conservación de *Cattleya quadricolor* Lindl. en el Valle del Cauca, Colombia

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*Cattleya quadricolor* es endémica a Colombia, presente en solo 3 departamentos (Valle del Cauca, Quindío y Risaralda), categorizada como EN en el libro rojo de plantas de Colombia (Calderon *et al.*, 2007), y es una de las 22 especies de flora con mayor amenaza en el Valle del Cauca. Con este estudio, se generó el mapa de distribución actual y potencial de *C. quadricolor*, se evaluaron las condiciones ecológicas y demográficas en su hábitat, y se identificaron las amenazas y fuentes de presión para su plan de manejo. Un total de 3.000 m<sup>2</sup>, fueron medidos, distribuidos en parcelas de 50 m × 2 m, en los que árboles y arbustos con DAP mayor o igual a 2,5 cm que hospedaran *C. quadricolor* fueron censados. Se contaron los individuos sobre los forófitos detallando, estado reproductivo, número de pseudobulbos, altura sobre el forófito, ángulo, posición del forófito, entre otros. Los resultados demuestran la presencia de la

especie en 16 municipios con un área de extensión de presencia de 234.359 Has. comprendidas entre los 930 y 1.450 msnm. Se demostró que *C. quadricolor* es más abundante al interior del bosque (55%) que en árboles aislados (16%) y sus densidades oscilan entre de 260 a 1.180 Ind/Ha. *Cattleya quadricolor* crece sobre 20 tipos de forófitos, sin embargo 4 de ellos *A. excelsum* (37%), *G. ulmifolia* (26%), *F. insipida* (12%) y *E. ulei* (7%) y representan el 82% de la preferencia de esta especie. Se concluye que la estrategia de conservación *in-situ* debe incluir un incremento de la cantidad y la calidad del hábitat a través del manejo de sus cuatro principales forófitos, la consideración de las variables aquí medidas, el desarrollo de un protocolo de propagación *in vitro* para su reintroducción, así como la disposición de exedentes en viveros comerciales para restar presión en campo.

## Characterization of *Brassolaeliocattleya* Raye Holmes 'Mendenhall' - putatively transformed for resistance to Cymbidium mosaic virus

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Orchids are infected by more than 50 different viruses. Infected plants bloom less efficiently, lack vigor, and produce lower-quality flowers than healthy plants. One of the most important viruses, with worldwide distribution, is Cymbidium mosaic virus (CyMV). It is a single-stranded, RNA virus belonging to the group of rod-shaped potexviruses. In previous

research, a *Brassolaeliocattleya* orchid was transformed with the coat protein gene from Cymbidium mosaic virus using an *Agrobacterium*-mediated method. The aim was to improve resistance of the orchid to CyMV. Protocorm-like bodies of *Brassolaeliocattleya* Raye Holmes 'Mendenhall' were used. A full-length coat protein gene of CyMV was cloned into a vector, which

also contained kanamycin resistance for the selection of transformants. The coat protein was inserted under control of a *CaMV 35S* promoter in an anti-sense orientation. Putative transformants were selected for four months on media containing 25 µg/mL Geneticin Disulfate (G418). The objective of the present study is to further investigate and characterize the putative

transgenic lines, which have been selected for antibiotic resistance. Molecular characterization of transformants is ongoing. Micropropagation is being carried out to multiply the transformed plant material. Plantlets will be used to develop an *in vitro* inoculation assay to test the resistance to CyMV. Future work will investigate the resistance of these lines to the Cymbidium mosaic virus.

## Pestiferous scale insects on native epiphytic orchids in south Florida: a new threat posed by exotic species

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In 2009, phytophagous insects (Hemiptera: Coccidae, Pseudococcidae) were collected from the inflorescences of the ghost orchid, *Dendrophylax lindenii*, at an isolated natural site in Collier Co., Florida, surrounded by an urban area. During the next two years, additional surveys were carried out in more remote orchid-rich habitats to determine if, and to what extent, other native epiphytic orchids were infested. Within the Florida Panther National Wildlife Refuge in 2010, 29 of 46 orchids sampled at one site (Cochran Lake) harbored three species of exotic scales: orchid pit scale (*Asterolecanium epidendri* Asterolecaniidae), brown soft scale (*Coccus hesperidum*, Coccidae), and boisduval scale (*Diaspis boisduvalii*, Diaspididae). Heaviest infestations were noted on *Epidendrum amphotomum*, *E. nocturnum*, *E. rigidum*, and *Prosthechea cochleata*.

The follow-up study the second year (2011) was then expanded to include Fakahatchee Strand State Preserve. A total of 1,726 orchids spanning 10 taxa were surveyed at seven locations. Boisduval scale was detected on 2.3% of the orchids from six of the ten orchid species in both the Florida Panther NWR and Fakahatchee Strand. *Prosthechea cochleata* and *E. amphotomum* appeared to be most vulnerable to this scale, with infection totals of 5.8% and 2.1%, respectively. Of 44 scales collected from the 39 orchids, 27% hosted hymenopteran parasitoids in various stages of development. The presence of *D. boisduvalii* adds an additional burden to state-endangered orchid populations and indicates that resource managers may need to expand management approaches to include plant-parasitic insect control.





