INVITED PAPERS FROM THE

V SCIENTIFIC CONFERENCE ON ANDEAN ORCHIDS

Cali, Colombia, Pontificia Universidad Javeriana
19–21 November 2015

EDITED BY

NICOLA S. FLANAGAN
Pontificia Universidad Javeriana, Cali, Colombia

ALEC M. PRIDGEON
Royal Botanic Gardens, Kew, U.K.

and

FRANCO PUPULIN
Universidad de Costa Rica, Jardín Botánico Lankester

LANKESTERIANA

LANKESTERIANA 16(2): 189–200. 2016. **doi**: http://dx.doi.org/10.15517/lank.v16i2.26005

EVOLUTIONARY DIVERSIFICATION AND HISTORICAL BIOGEOGRAPHY OF THE ORCHIDACEAE IN CENTRAL AMERICA WITH EMPHASIS ON COSTA RICA AND PANAMA

DIEGO BOGARÍN[1,2,3,7], FRANCO PUPULIN[1], ERIK SMETS[3,4,5] & BARBARA GRAVENDEEL[3,4,6]

[1] Lankester Botanical Garden, University of Costa Rica, Cartago, Costa Rica
[2] Herbarium UCH, Autonomous University of Chiriquí, Chiriquí, Panama
[3] Naturalis Biodiversity Center, Leiden University, Leiden, The Netherlands
[4] Institute Biology Leiden, Leiden University, Leiden, The Netherlands
[5] KU Leuven, Ecology, Evolution and Biodiversity Conservation, Leuven, Belgium
[6] University of Applied Sciences Leiden, Leiden, The Netherlands
[7] Author for correspondence: diego.bogarin@ucr.ac.cr

ABSTRACT. Historically, the isthmus of Costa Rica and Panama has been a source of fascination for its strategic position linking North America to South America. In terms of biodiversity, the isthmus is considered one of the richest regions in the world. Orchidaceae is the most diverse plant family in the area, and the number of species is triple that of other well-represented angiosperm families such as Rubiaceae, Fabaceae and Poaceae. Though we are still far from knowing the exact number of orchid species occurring extant in both countries nowadays, at present the orchid flora reported for Costa Rica (1574 spp.) and Panama (1372 spp.) summarise together about 2010 species; which represents 6.5-8.0% of all orchid species on just about 1% of the Earth's land surface. Pleurothallidinae and Laeliinae are the most species rich groups and contain the largest genera: *Lepanthes*, *Pleurothallis*, *Stelis and Epidendrum*. These groups significantly outnumber the other genera recorded in terms of species richness. Some factors explaining this regional taxonomic diversity of orchids are the natural land bridge uniting three of the 25 recognized hotspots worldwide (Mesoamerica, Choco/Darien/Western Ecuador and tropical Andes), the climatic influence of the Pacific and Atlantic oceans, and the recent lifting of the Cordillera de Talamanca and the formation of foothills of Majé, Darien and San Blas in Panama and western Colombia. Although these factors can explain the high diversity in general terms, detailed information is needed to understand species diversification as well as the evolution of the floristic composition. Updated floristic inventories (yielding a rate of 25 new species/year) and the study of biological mechanisms that have led to the evolutionary diversification of *Lepanthes* (one of the major groups of orchids) are the main ongoing research projects to elucidate the evolution of Orchidaceae in Costa Rica and Panama. Towards this end, we present some preliminary results of the research conducted in this direction including the integration of phylogenetics, pollination ecology, taxonomy and biogeography.

RESUMEN: Históricamente, el Istmo de Costa Rica y Panamá ha sido fuente de fascinación por su posición estratégica, uniendo América del Norte con América del Sur. En términos de biodiversidad, el Istmo es considerado una de las regiones más ricas del mundo. Orchidaceae es la familia de plantas más diversa en la zona, y el número de especies triplica a otras familias de angiospermas bien representadas como Rubiaceae, Fabaceae y Poaceae. Aunque todavía estamos lejos de conocer el número exacto de especies de orquídeas existentes en ambos países, en la actualidad la flora de orquídeas registradas para Costa Rica (1574 spp.) y Panamá (1372 spp.) suman en conjunto alrededor de 2010 especies; lo que representa 6,5-8,0% de todas las especies de orquídeas en apenas alrededor de 1% de la superficie terrestre del planeta. Pleurothallidinae y Laeliinae son los grupos más ricos en especies y contienen la mayor géneros: *Lepanthes*, *Pleurothallis*, *Stelis* y

Received 3 May 2016; accepted for publication 19 July 2016. First published online: 30 August 2016.

Epidendrum. Estos grupos superan significativamente los otros géneros registrados en términos de riqueza de especies. Algunos de los factores que explican esta diversidad taxonómica regional de orquídeas son el puente natural que une tres de los 25 puntos reconocidos en todo el mundo (Mesoamérica, Chocó / Darién / Ecuador Occidental y Andes tropicales), la influencia climática de los océanos Pacífico y Atlántico, y la reciente elevación de la Cordillera de Talamanca y la formación de estribaciones de Majé, Darién y San Blas, en Panamá y el oeste de Colombia. Aunque estos factores pueden explicar la alta diversidad de orquídeas en términos generales, se necesita información detallada para comprender la diversificación de especies, así como la evolución de la composición florística. Los inventarios florísticos actualizados (con una tasa de 25 nuevas especies / año) y el estudio de los mecanismos biológicos que han llevado a la diversificación evolutiva de *Lepanthes* (como uno de los géneros más grandes de orquídeas) son los principales proyectos de investigación en curso para dilucidar la evolución de Orchidaceae en Costa Rica y Panamá. Con este fin, presentamos algunos resultados preliminares de la investigación llevada a cabo en esta dirección que incluye la integración de estudios filogenéticos, ecología de la polinización, taxonomía y biogeografía.

Key words / Palabras clave: biodiversity, floristics, pollination ecology, *Lepanthes* / biodiversidad, florística, la polinización ecología, *Lepanthes*

Introduction. Historically, the isthmus of Costa Rica and Panama has been a source of fascination for its strategic position linking North America to South America. The geological events that led to the closure of the isthmus that started with the formation of a volcanic arc dating fromlatest Cretaceous to Eocene, 67 to 39 million years ago (Mya) (Montes *et al.* 2015) have been studied extensively but are still controversial. There is no consensus about when the isthmus closed completely the Central American Seaway (CAS) separating the Pacific from the Atlantic Ocean and favoring the Great American Biotic Interchange (GABI). Traditionally this time, however, was established between 3.5-5.0 Mya, but other studies that include new information suggest a closure between 13-15 Mya in the middle Miocene (Bacon *et al.* 2015, Montes *et al.* 2015). Despite this, with the initial emergence of a volcanic arc in the Cretaceous, orchids had time to colonize some of these oceanic islands by wind dispersal of seeds and evolve there.

According to a phylogenomic analysis and net diversification regimes across lineages using BAMM analysis, Givnish *et al.* (2015) proposed that Orchidaceae arose around 112 Mya in the Cretaceous, long before the formation of the arc and subsequent closure of the Isthmus of Panama. However, the

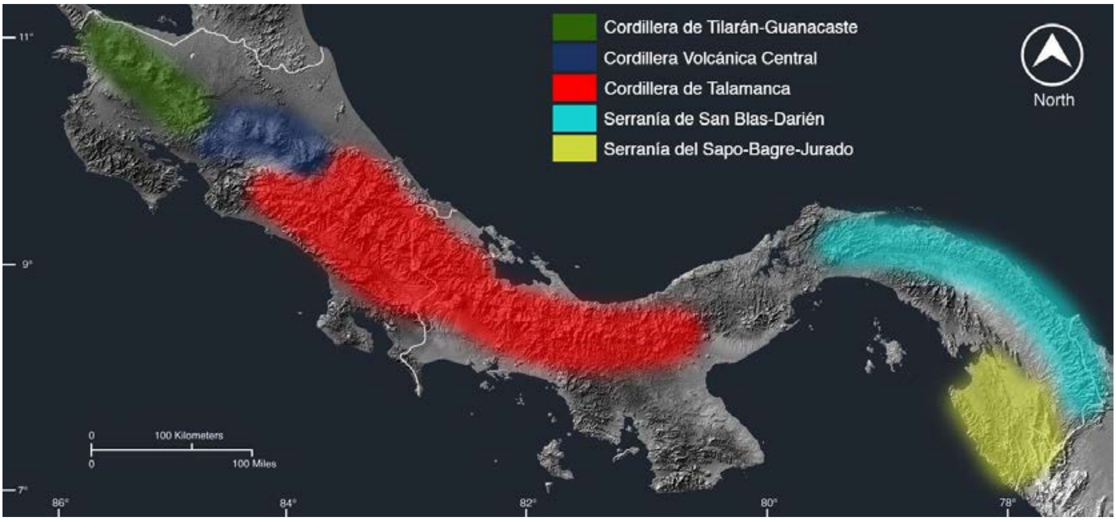


Figure 1. Geography of southern Central America (Costa Rica and Panama) showing the main ranges: Talamanca crossing both countries and San Blas-Darien on the southeast of Panama towards Colombia.

most diverse Neotropical subtribes: Laeliinae, Oncidiinae, Maxillariinae and Pleurothallidinae probably diversified between 10-25 Mya after the last acceleration of net diversification rate that occurred about 25 Mya, overlapping with the possible closure of the Isthmus proposed recently (Bacon *et al.* 2015, Givnish *et al.* 2015, Montes *et al.* 2015). Indeed the flora of the Isthmus is dominated mainly by species of Laeliinae and Pleurothallidinae that diversified about 18 Mya. Analyzing the current floristic composition of the species in Costa Rica and Panama (Fig. 1) we could find general patterns that help to understand better the evolutionary and biogeographic relationships among other geologically older regions such as the Andes, northern Central America and the Antilles.

Table 1. Number of genera, species and endemics (%) in Costa Rica and Panama.

	Costa Rica	Panama	Total
Genera	199	187	211
Species	1574	1372	2012
Endemics	485 (30.8)	299 (21.8)	784

Orchidaceae diversity in Costa Rica and Panama. Updated floristic inventories in Costa Rica and Panama (both countries treated as a biogeographic unit) contain some 2012 species of orchids of which 934 are shared (Bogarín *et al.* 2014b, Bogarín in prep.). From these figures, 784 (39%) species are endemic to the Isthmus (Table 1).

A strategy to analyze the current evolutionary and

Table 2. The most diverse genera in Costa Rica and Panama. Number of species and percentage relative to the total of species of each genus.

Costa Rica		**Panama**	
Genus	Number of species (%)	Genus	Number of species (%)
Epidendrum	207 (13.8)	*Epidendrum*	221 (14.7)
Stelis	88 (11.0)	*Lepanthes*	151 (13.5)
Lepanthes	66 (5.9)	*Stelis*	103 (12.9)
Pleurothallis	54 (6.8)	*Camaridium*	48 (32.0)
Camaridium	48 (32.0)	*Pleurothallis*	48 (6.0)
Scaphyglottis	39 (35.5)	*Specklinia*	44 (44.0)
Sobralia	39 (17.7)	*Scaphyglottis*	38 (34.5)
Specklinia	34 (34.0)	*Sobralia*	38 (17.3)
Oncidium	32 (8.0)	*Telipogon*	37 (14.8)
Dichaea	26 (26.0)	*Masdevallia*	34 (3.8)

Table 3. Percentage of the number of endemic species and the total of species of the genera with most endemic species in Costa Rica and Panama

Costa Rica		**Panama**	
Genus	Endemic species (%)	Genus	Endemic species (%)
Telipogon	83.8	*Telipogon*	73.9
Lepanthes	67.5	*Pleurothallis*	42.6
Sobralia	39.5	*Sobralia*	42.6
Masdevallia	38.2	*Masdevallia*	34.6
Epidendrum	36.2	*Lepanthes*	31.5
Stelis	35.9	*Stelis*	33.3
Specklinia	31.8	*Epidendrum*	23.1
Pleurothallis	31.3	*Camaridium*	16.7
Camaridium	29.2	*Specklinia*	20.6

FIGURE 2. Some representatives of the major groups of Orchidaceae present in Costa Rica and Panama. From left to right: *Camaridium campanulatum*, *Epidendrum nocturnum*, *Epidendrum* (*Oerstedella*) *wallisii*, *Lepanthes matamorosii*, *L. bradei*, *Pleurothallis* sp., *Scaphyglottis pulchella*, *Stelis transversalis*, *Telipogon panamensis*.

floristic relationships of the Orchidaceae of the Isthmus is the study of the most diverse groups in the region. An analysis of the various genera occurring in both Costa Rica and Panama shows that *Epidendrum* L., *Lepanthes* Sw. and *Stelis* s.s. Sw. contain the most species and the highest rates of endemism (Tables 2,3). These genera are also monophyletic (Pridgeon *et al.* 2001) and therefore there is no bias due to the use of different nomenclatural circumscriptions that might cause variations in the number of species assigned to a genus.

One factor that can affect the interpretation of evolutionary and biogeographic data is the intensity of the alpha-taxonomic work. The most diverse genus in the Isthmus is *Epidendrum,* the taxonomy of which has been developed in detail and consistently by Hágsater and colleagues. *Lepanthes* is a diverse genus, and despite extensive work by Luer (2003a) there are still many species to be revealed, especially in the Cordillera de Talamanca and Panama. If this trend continues, *Lepanthes* may exceed *Epidendrum* in number of species recorded in the Isthmus (Pupulin & Bogarín, 2014). Moreover, the taxonomy of *Stelis* s.s. is the least developed, and the conclusions based on these data are likely biased (Luer, 2003b). Botanical exploration and alpha-taxonomy are therefore tasks that must be promoted with impetus in the region.

Some other diverse groups in the Isthmus are *Camaridium* Lindl., *Dichaea* Lindl., *Oncidium* Sw., *Pleurothallis* R.Br., *Scaphyglottis* Poepp. & Endl., *Sobralia* Ruiz & Pav., *Specklinia* Lindl. and *Telipogon* Kunth. (Fig. 2) These groups also maintain a tendency to hold many endemic species. The taxonomic work in these genera has also revealed new species and expanded geographic distributions, encouraging more potential case studies to understand the evolution and diversification of Orchidaceae in the Isthmus (Pupulin *et al.* 2012, Bogarín *et al.* 2014a, Dressler & Pupulin 2015).

Biogeography and endemism in Costa Rica and Panama. About 40% of the species are endemic to the Isthmus. The highest rate of endemism recorded could be related to geological events of volcanic arc and vicariance phenomena produced by the lifting of the Cordillera de Talamanca. Allopatric speciation in *Lycaste bruncana* Bogarín and *L. tricolor* Rchb.f. (Fig. 3) — among other examples also found in *Brassia* R.Br., *Epidendrum*, *Kefersteinia* Rchb.f., *Oncidium*, *Pleurothallis* and *Stelis* — indicate an important role of the altitudinal division produced by Talamanca and its climate barrier effect blocking the Caribbean tradewinds (Pupulin 2001, Bogarín 2007, Pupulin & Bogarín 2012).

The highest rates of endemism are found in the most diverse genera. For example, 90% of the species of *Lepanthes* are endemic and about 50% of the species of *Stelis* and *Epidendrum* (Table 4). The study of the factors favoring this high endemism in *Lepanthes* is key to understanding its diversification and will be discussed later as a study model group. Other genera attract attention because, although not as diverse, they show high rates of endemism; one of these is *Telipogon*, in which more than 70% of species are endemic. Current floristic relationships with other groups of orchids of the Andes is evident. *Telipogon* is a diverse genus in the highlands of the Isthmus, and its northern distribution is limited (Williams, Whitten, & Dressler, 2005). Other genera of South American affinities are *Brachionidium* Lindl., *Fernandezia* Lindl. and *Pterichis* Lindl. that almost reach their northernmost distribution in the Cordillera de Talamanca (Bogarín *et al.* 2014b).

Table 4. The most diverse genera and the % of endemic species in Costa Rica and Panama

	Species in the Isthmus	**% endemic species**
Lepanthes	155	90.12
Epidendrum	133	46.18
Stelis	60	43.80

About 10 genera are present in Panama but not in Costa Rica. These genera have a strong South American relationship: *Discyphus* Schltr., *Eloyella* P.Ortiz, *Koellensteinia* Rchb.f., *Neomoorea* Rolfe, *Rudolfiella* Hoehne and *Selenipedium* Rchb.f. They range from Central Panama to the southeast of Darien and towards Colombia, indicating a common geological history of this area but different from western Panama and southeast Costa Rica. The geological formation of foothills of Maje, Darien and San Blas in Panama and western Colombia is reflected in our species composition data. Because of its [antecedent? To what does this refer?] geographical distribution, it is likely that species in *Dinema* Lindl., *Euryblema* Dressler, *Helleriella* A.D. Hawkes and *Horichia* Jenny can be recorded with high probability in Costa Rica (Bogarín *et al.* 2014b).

On the other hand 18 genera are present in Costa Rica and are still not registered in Panama. Some of them have mostly a northern influence such as *Arpophyllum* La Llave & Lex. and *Restrepiella* Garay & Dunst. However, it is likely that *Epistephium* Kunth, *Funkiella* Schltr., *Lankesterella* Ames, *Trevoria* F.Lehm., *Tropidia* Lindl. and *Warmingia* Rchb.f. with representatives in South America are distributed in Panama. The bias resulting from less floristic and alpha-taxonomic work in Panama should be reduced in the upcoming years (Bogarín *et al.* 2013).

Evolutionary diversification and floristic composition of Costa Rica and Panama. Our focus is on studying the factors that led to the formation of the current species composition of Orchidaceae in the Isthmus. Based on our taxonomic experience we

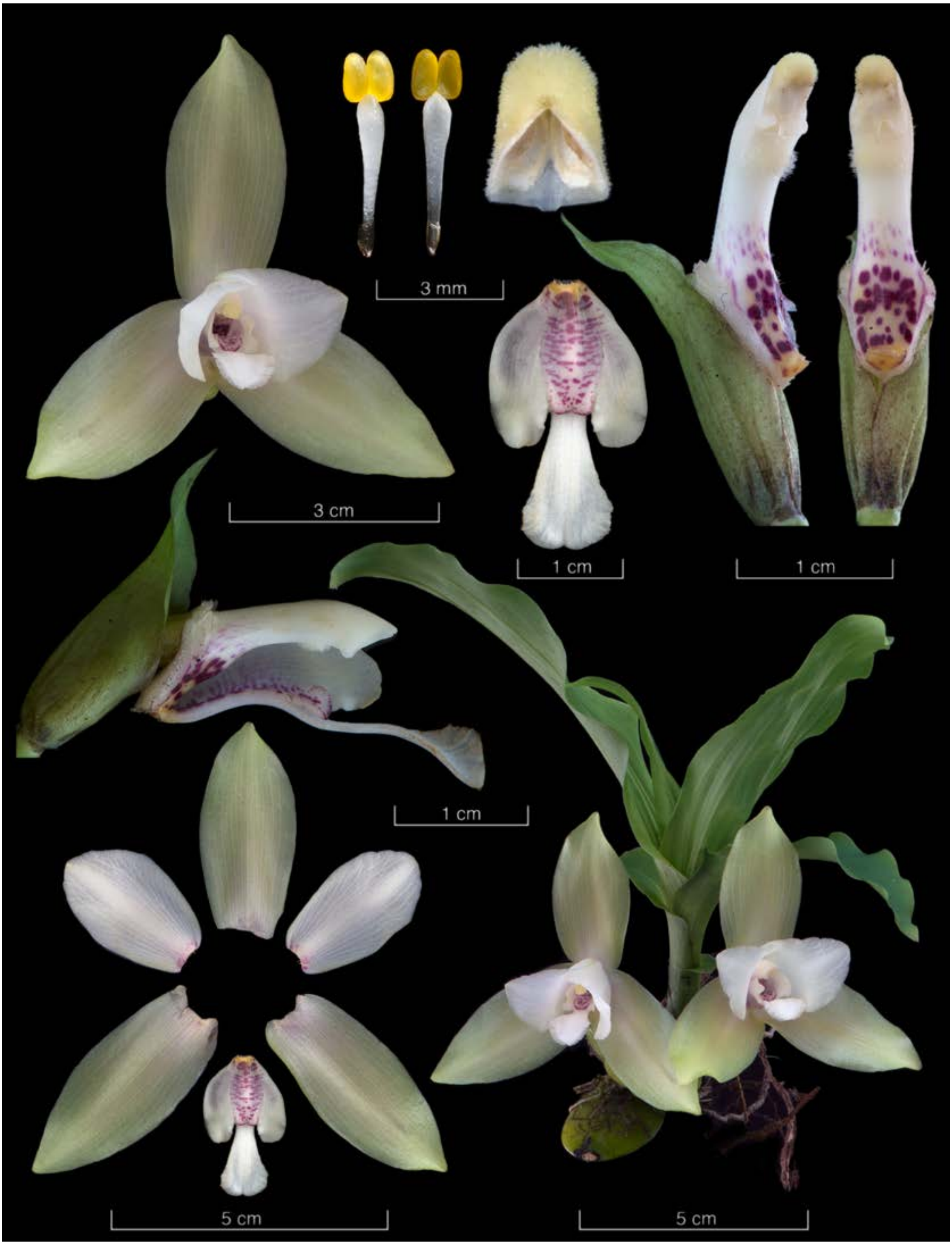


Figure 3. Lankester Composite Digital Plate of *Lycaste bruncana,* a species from Costa Rica and Panama restricted to the Pacific watershed of Cordillera de Talamanca.

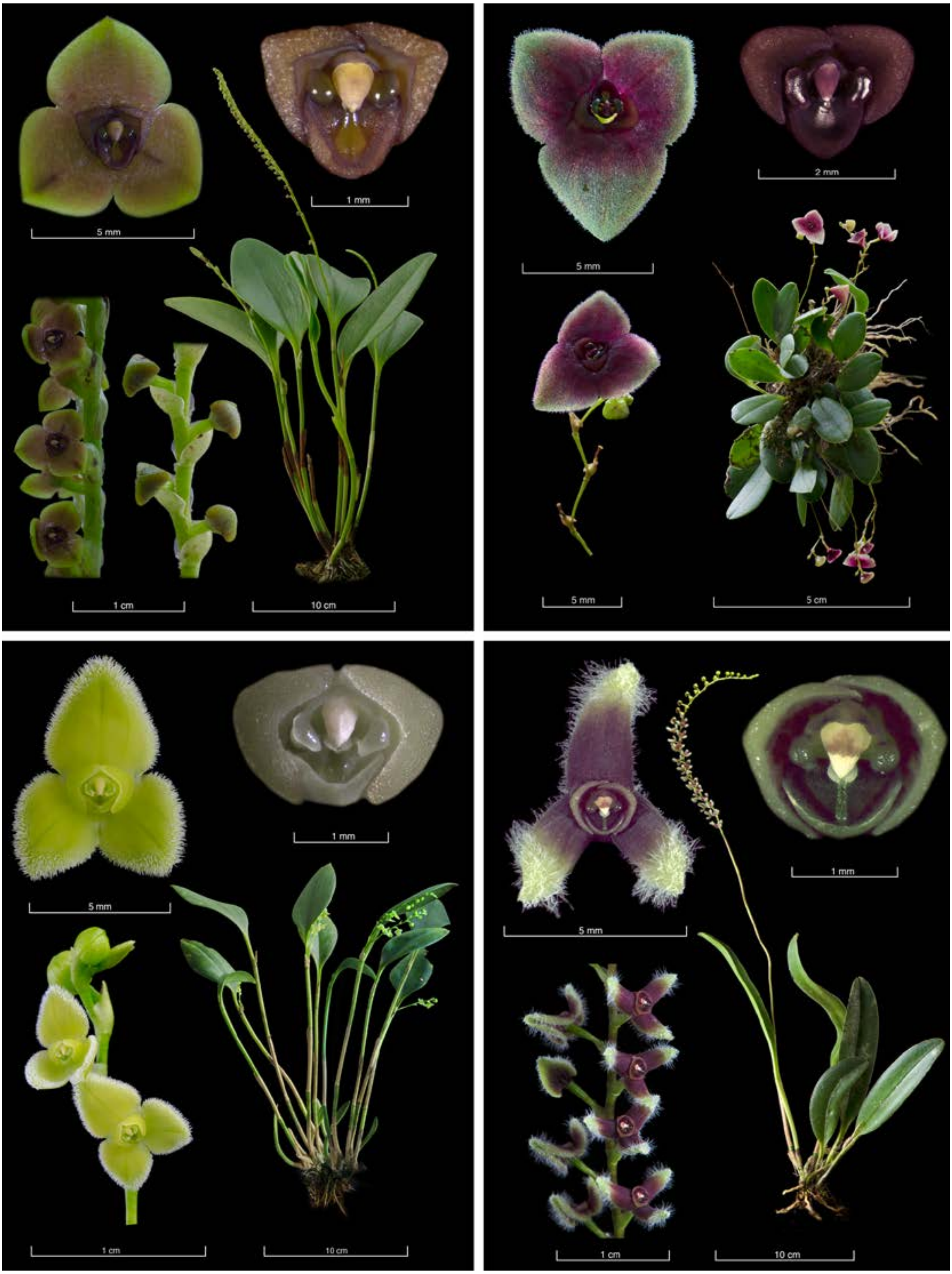


Figure 4. Some species of the highly diverse genus *Stelis* from Costa Rica and Panama. Species are currently under taxonomic review.

Figure 5. Species of Cecydomiididae carring a pollinarium of *Stelis* cf. *parvula*.

have selected *Lepanthes* and closely related genera as a model group to study the extraordinary species richness and evolution in Costa Rica and Panama and its relationship with the Andean flora. We intend in the future to extend this model to other diverse groups such as *Stelis*. *Epidendrum* is another interesting group, and it is being evaluated by Hágsater and co-workers so there will be information available in the future. Although *Stelis* s.s. remains an excellent group as a candidate to study their high evolutionary diversification, the limited taxonomic expertise and little ecological information prevents us to address this group (Fig. 4). However, some clues about its pollination mechanism (hitherto little-known) indicate that it may be pollinated by gall midges of Cecidomyiididae under conditions that we are still exploring (Fig. 5).

The genus *Lepanthes*. *Lepanthes* is one of the major genera in the Pleurothallidinae. With over 1000 spp., the genus ranges from southern Mexico and the Antilles to Peru and Bolivia, with few species in the Guianas and Brazil. Plants grow mostly from 1500 to 3000 m elevation in humid, often shady places. Highest diversity is found in the Andean region of Colombia and Ecuador with more than 300 species in each country (Luer 1996, Luer & Thoerle 2012) (Fig. 6). *Lepanthes* is represented in Costa Rica and Panama with about 150 spp. Only two species are shared with Colombia and Ecuador. This may reflect the floristic influence of the Andean region in Costa Rica and Panama at the genus level but not the species level. Species are usually restricted to specific ranges or mountains, and endemism is high.

Plants are recognized by the monophyllous ramicauls, enclosed by a series of lepanthiform sheaths and congested, distichous inflorescences. Floral morphology distinguishes *Lepanthes* from other genera with lepanthiform sheaths (*Draconanthes* (Luer) Luer, *Trichosalpinx* Luer and *Lepanthopsis* (Cogn.) Hoehne among others). Flowers are characterized by the ovate to elliptic sepals and the transversely bilobed petals. Lip morphology is complex (Fig. 7). It is usually bilaminate with the two blades supported by connectives that often lift the blades above the column. The central part of the lip is made up by the body, which is attached to the column. The appendix is developed from the sinuous between the connectives andvaries morphologically among the species in different combinations of lobes, hairs, projections, trichomes and membranes. Although *Lepanthes* has been poorly sampled phylogenetically, it is considered a monophyletic group (Pridgeon, Solano-Gómez & Chase, 2001). Our preliminary phylogenetic analysis support the monophyly of *Lepanthes*. It is important to note that the genus *Neooreophilus* Archila, which is closely related to *Andinia* (Luer) Luer and not to *Lepanthes,* seems to be a case of convergence (Fig. 8). *Neooreophilus* species have a similar flower morphology as *Lepanthes,* and there is some evidence of its pollination by pseudocopulation (S. Vieira-Uribe, pers. comm. 2015). *Neooreophilus* is absent in Mesoamerica, and it might be a younger group when compared to *Lepanthes,* which is widespread in the Neotropics. Phylogenetics of these two groups will help to shed light on this hypothesis.

The floral morphology of *Lepanthes* varies astonishingly around the same scheme in all the >1000 species known. Petals and lip tend to be reduced or almost absent in some species. Flowers are developed above or beneath the leaves or sometimes in inflorescences surpassing the leaves. The most common colors of flowers are yellow, red, orange, purple (rarely green) or a combination of these. The appendix plays an important role in pollination of *Lepanthes* flowers. Blanco & Barboza (2005) described the first case of pseudocopulation in the genus. Males of a fungus gnat, *Bradysia floribunda* (Diptera: Sciaridae), visit flowers of *L. glicensteinii* Luer, apparently attracted by sexual

FIGURE 6. Some species of *Lepanthes* from Costa Rica and Panama. Species are mostly endemic and show a wide range of morphological variation around the same scheme. Note the coloration of the flowers, which might be involved in attraction of pollinators.

pheromone-like scents. The male adheres to the flower appendix during its attempt copulation. In this process the insect removes the pollinarium with the abdomen. Calderon-Sáenz (2012) observed the same phenomenon in *L. yubarta* E.Calderon visited by a species of *Bradysia* in Valle del Cauca, Colombia. Sciaridae flies, commonly known as dark-winged fungus gnats, are a diverse group of insects with more than 8000 species worldwide. Eggs are deposited between the lamina of sporocarps of fungi, and the larvae feed on sporocarps and other decaying organic matter such as rotten trunks or plant roots or leaves. Some species are pests of important economic crops such as mushrooms. Blanco & Barboza (2005) and Calderon-Sáenz (2012) clearly described the pollination of *Lepanthes* but left many evolutionary questions unanswered. We are studying more cases of pollination in other *Lepanthes* species where morphological evidence indicates that other parts of the body of insects are being used such that the pollinia are not always attached to the abdomen. It is

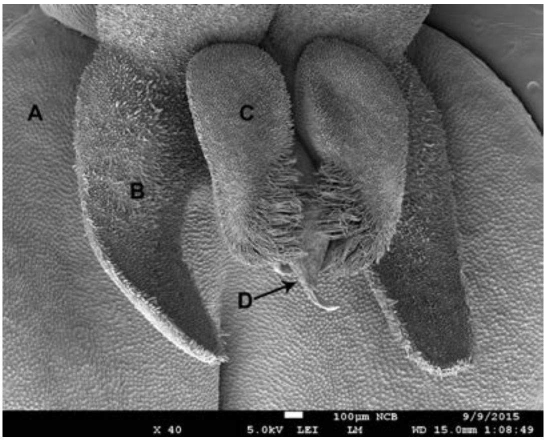


FIGURE 7. Scanning electron microscopy (SEM) of a flower of *Lepanthes horichii* showing the complex morphology in detail. A. Sepal., B. Petal (lower lobe)., C. Lip (lobe)., D. Column showing the apical anther.

likely that production of pheromone-like compounds are the sources of attraction. We have evidence that flowers use this strategy to attract males. The anatomy of the flower is being studied in order to find possible secretory structures involved in pollinator attraction (Fig. 7).

Sciarids are attracted by yellow colors. Special traps are designed to catch flies in greenhouses made up by yellow cardboard and petroleum jelly. Although this method proved to be less effective in studying *Lepanthes* pollination (Godden 2002), the approach needs to be used in large populations to increase the probabilities of catching flies with pollinia.

Sciarid flies have short life cycles (Wilkinson & Daugherty, 1970). Adults usually live less than 7 days, and they are considered poor flyers. Thus the chance to deceive the inexperienced males may be high. Sciaridae is a highly diverse group but poorly known. Indeed, the pollinator of *L. glicensteinii* observed by Blanco & Barboza (2005) was an undescribed species. The behavior and natural history of Sciaridae are key to understanding the evolution of *Lepanthes*.

Why is *Lepanthes* more diverse than closely related genera such as *Anathallis* Barb.Rodr., *Draconanthes, Lankesteriana* Karremans, *Lepanthopsis, Trichosalpinx* and *Zootrophion* Luer? A hypothesis is that pseudocopulation triggered the high speciation levels in *Lepanthes*. To study the evolutionary diversification of *Lepanthes* and the possible triggers of speciation, it will be necessary to extend the molecular phylogenetic sampling of the "*Lepanthes*- clade" as described by Pridgeon, Solano-Gómez & Chase (2001) in order to find answers to the evolutionary success of *Lepanthes* as compared to its sister genera. However, the pollination mechanisms that operate in those sister genera are also important for comparisons with *Lepanthes*. Preliminary observations on the pollination of *Trichosalpinx* revealed a frequent visitation by biting midges of the Ceratopogonidae family. The taxonomy of *Trichosalpinx* has been treated by Luer (2003c) and more recently by Fernández (2013), and systematic studies of *Lepanthopsis*, *Anathallis*, *Lankesteriana* and *Zootrophion* are underway. *Lepanthes* taxonomy has been studied by Luer & Thoerle (2012) and particularly in Costa Rica and Panama by Luer & Dressler (1986), Luer (2003a), Pupulin, Bogarín & Jiménez (2009) and Pupulin & Bogarín (2014).

Givnish *et al.* (2015) pointed out that all factors such as the role of limited dispersal of seeds and ineffective pollinators, limited gene flow, population

Figure 8. Floral convergence among the species of *Neooreophilus* (A, B) and *Lepanthes* (C).

bottlenecks and genetic driftdeserve to be studied, and *Lepanthes* is one of the best study cases for that. We also propose the delimitation of biogeographical areas within Costa Rica and Panama in order to draw accurate conclusions about endemism and species distribution. It would be advisable that some of the topics discussed here include the Andean flora, which is particularly rich in these groups of orchids. This study has future conservation applications because the major groups of orchids and associated pollinators and hosts are vulnerable to climate change in cold, high-elevation areas where they are most diverse.

Acknowledgments. We acknowledge the staff of Lankester Botanical Garden University of Costa Rica and the Naturalis Biodiversity Center. The Vice-Presidency of Research of the University of Costa Rica provided support through the project "Filogenia, diversificación evolutiva y biogeografía histórica de los principales grupos de orquídeas en la Baja Centroamérica (Costa Rica y Panamá) con énfasis en *Lepanthes* y géneros cercanos (Pleurothallidinae)" (814-B6-140). We thank the Costa Rican Ministry of Environment and Energy (MINAE) and its National System of Conservation Areas (SINAC) for issuing the Scientific Passports under which wild species treated in this study were collected. The organizers of the Fifth Scientific Conference on Andean Orchids for the provided facilities for the event. Jerry Harrison and two anonymous reviewers made helpful comments on the manuscript. This work is part of the Ph.D. project of the first author, titled "Evolutionary diversification and historical biogeography of the Orchidaceae in Central America with emphasis on Costa Rica and Panama," at the University of Leiden, The Netherlands and Naturalis Biodiversity Center.

Literature cited

Bacon, C. D., Silvestro, D., Jaramillo, C., Smith, B.T., Chakrabarty, P. & Antonelli, A. (2015). Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceedings of the National Academy of Sciences*, 112, 6110–6115.

Bogarín, D. (2007). A new *Lycaste* (Orchidaceae: Maxillariinae) from Costa Rica. *Lankesteriana* 7(3) : 543-549.

Bogarín, D., Pupulin, F., Arrocha, C. & Warner, J. (2013). Orchids without borders: studying the hotspot of Costa Rica and Panama. *Lankesteriana* 13(1–2): 13–26.

Bogarín, D., Serracín, Z. & Samudio, Z. (2014a). Illustrations and studies in Neotropical Orchidaceae. The *Specklinia condylata* group (Orchidaceae) in Costa Rica and Panama. *Lankesteriana* 13(3): 185–206.

Bogarín, D., Serracín, Z., Samudio, Z., Rincón, R. & Pupulin, F. (2014b). An updated checklist of the Orchidaceae of Panama. *Lankesteriana* 14(3): 135–364.

Blanco, M.A. & Barboza, G. (2005). Pseudocopulatory pollination in *Lepanthes* (Orchidaceae: Pleurothallidinae) by fungus gnats. *Annals of Botany* 95: 763–772.

Calderón-Sáenz, E. (2012). Remoción de polinarios de *Lepanthes yubarta* por una mosca fungosa del género *Bradysia*, bajo condiciones seminaturales. *Orquideología* XXIX(1): 31–-34.

Godden, G.T. (2002). Pollination and speciation of *Lepanthes*: an approach to understanding orchid evolution. *Pleurothallid News and Views* 14: 52–54.

Dressler, R.L. & Pupulin, F. (2015). *Sobralia lentiginosa* (Orchidaceae: Sobralieae). An attractive new species from Costa Rica. *Lindleyana in Orchids* (*Bull. Amer. Orch. Soc.)* 84(6): 374–376.

Fernández, M. (2013). *Tubella* - die etwas anderen *Trichosalpinx*. *Tubella*: Those other *Trichosalpinx*. *Die Orchidee* 64(4): 310–317

Givnish, T. J, Spalink, D., Ames, M., Lyon, S.P., Hunter, S.J., Zuluaga, A., Iles, W. J., Clements, M. A., Arroyo, M.T., Leebens-Mack, J., Endara, L., Kriebel, R., Neubig, K.M., Whitten, W.M., Williams, N.H., Cameron, K.M. (2015). Orchid phylogenomics and multiple drivers of their extraordinary diversification. *Proceedings of the Royal Society B: Biological Sciences* 282(1814). doi: 10.1098/rspb.2015.1553.

Luer, C.A. (1996). Icones Pleurothallidinarum, XIV: The genus *Lepanthes*, subgenus *Lepanthes* in Ecuador. *Monographs in Systematic Botany from the Missouri Botanical Garden* 61: 1–255.

Luer, C.A. (2003a). *Lepanthes*. In: B. E. Hammel, M.H. Grayum, C. Herrera & N. Zamora (Eds.), *Manual de Plantas de Costa Rica* Vol. 3. (pp. 216-255) Monocotiledóneas (Orchidaceae-Zingiberaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* 93: 1–884.

Luer, C.A. (2003b). *Stelis*. In: B. E. Hammel, M.H. Grayum, C. Herrera & N. Zamora (Eds.), *Manual de Plantas de Costa Rica* Vol. 3. (pp. 521-245) Monocotiledóneas (Orchidaceae-Zingiberaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* 93: 1–884.

Luer, C.A. (2003c). *Trichosalpinx*. In: B. E. Hammel, M.H. Grayum, C. Herrera & N. Zamora (Eds.), *Manual de Plantas de Costa Rica* Vol. 3. (pp. 569-578) Monocotiledóneas (Orchidaceae-Zingiberaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* 93: 1–884.

Luer, C.A. & Thoerle, L. (2012). Icones Pleurothallidinarum XXXII: *Lepanthes* of Colombia. *Monographs in*

Systematic Botany from the Missouri Botanical Garden 123: 1–300.

Montes, C., Cardona, A., Jaramillo, C., Pardo, A., Silva, J.C., Valencia, V., Ayala, C., Pérez-Angel, L.C., Rodríguez-Parra, L.A., Ramírez, V., Niño, H. (2015). Middle Miocene closure of the Central American Seaway. *Science* 348(6231): 226–229.

Pridgeon, A.M., Solano-Gómez, R. & Chase, M.W. (2001). Phylogenetic relationships in Pleurothallidinae (Orchidaceae): combined evidence from nuclear and plastid DNA sequences. *American Journal of Botany* 88: 2286–2308. http://dx.doi.org/10.2307/3558390

Pupulin, F. (2001). Contributions to a reassessment of Costa Rican Zygopetalinae (Orchidaceae). The genus *Kefersteinia* Rchb.f. *Annalen des Naturhistorisches Museums Wien* 103B: 525–555.

Pupulin, F. & Bogarín, D. (2012). A new *Oncidium* from Costa Rica. *Lindleyana in Orchids* (West Palm Beach) 81(3): 176–179.

Pupulin, F. & Bogarín, D. (2014). *Aliae Lepanthes Machogaffenses* (Orchidaceae: Pleurothallidinae). *Harvard Papers in Botany* 19(2): 194–201.

Pupulin, F., Bogarín, D & Jiménez, D. (2009). New species and records in Mesoamerican *Lepanthes*. *Orchid Digest* 73: 136–145.

Pupulin, F., Karremans, A.P. & Gravendeel, B. (2012). A reconsideration of the empusellous species of *Specklinia* (Orchidaceae: Pleurothallidinae) in Costa Rica. *Phytotaxa* 63: 1–20.

Wilkinson, J.D. & Daugherty, D.M. (1970). The biology and immature stages of *Bradysia impatiens* (Diptera: Sciaridae). *Annals of the Entomological Society of America* 63(3):656–660.

Wilson, M. & Jost, L. (2011). Phylogenetic analysis of the Andean genus *Brachycladium* Luer (syn. *Oreophilus* Higgins & Archila) and closely related genera based on nuclear ITS sequencing. *Lankesteriana* 11(3): 370.

LANKESTERIANA 16(2): 201–218. 2016. **doi**: http://dx.doi.org/10.15517/lank.v16i2.26007

AN INTEGRATED STRATEGY FOR THE CONSERVATION AND SUSTAINABLE USE OF NATIVE *VANILLA* SPECIES IN COLOMBIA

Nicola S. Flanagan[1,2] & Ana Teresa Mosquera-Espinosa[1]

[1]Departamento de Ciencias Naturales y Matemáticas, Pontificia Universidad Javeriana-Cali, Colombia
[2] Author for correspondence: nsflanagan@javerianacali.edu.co

Abstract. The natural vanilla essence is obtained principally from the fruits of the species *Vanilla planifolia*, a member of the *Vanilla* aromatic clade, and native to the neo-tropics. Colombia is an important center of diversity for the genus with 22 *Vanilla* species reported, of which 18 belong to the aromatic clade. Colombian native *Vanilla* species comprise important genetic resources for the vanilla crop. Although there is no tradition of cultivation of vanilla in the country, these species have potential for establishment in sustainable agroforestry systems. Nonetheless, *Vanilla*, like many orchid species, is subject to both intrinsic and extrinsic conservation threats. This article outlines an integrated strategy for conservation, incorporating *in situ*, *ex situ* and *circa situm* measures to ensure the conservation of *Vanilla* species in Colombia, and to promote their sustainable use in community-based cultivation programs. This proposed strategy is also relevant for conservation managers in other countries with native *Vanilla* species.

Key words: *Vanilla* crop wild relatives, mycorrhizal fungi, *in situ*, *ex situ, circa situm* conservation, *Vanilla* Endophyte Collection

Introduction. Vanilla is one of the most economically important crops for low-altitude humid tropical regions, and is now cultivated globally. The natural fragrance of vanilla is a widely-used ingredient in both the food and cosmetic industries, and is obtained from the cured fruits of orchids in the genus *Vanilla* (Orchidaceae); principally the species *Vanilla planifolia* G. Jacks., and hybrid derivatives, native to the neo-tropics (Soto-Arenas 2003, 2006).

The vanilla crop is almost exclusively vegetatively-propagated, leading to low levels of genetic variation (Bory *et al.* 2008), and making the crop vulnerable to pathogen infestation. The fungal pathogen, *Fusarium oxysporum* f. sp. radicis-*vanillae* (Koyyappurath *et al.* 2015a), that causes root and stem rot is one of several with a severe impact on vanilla production systems (Hernandez-Hernandez 2011). Crop improvement programs to combat this susceptibility, as well as to incorporate further desirable traits in the development of new commercial vanilla cultivars are limited by a lack of germplasm from both the primary and the secondary gene pool (Roux-Cuvelier & Grisoni 2010). Despite the economic importance of this crop, it has received relatively little investigation, in particular with respect to the taxonomy, ecology and physiology of wild species in the genus.

Colombia is located at the center of the neotropical distribution for the aromatic *Vanilla* species, and recent studies indicate that a high diversity of *Vanilla* species is found in the country. This article aims to outline the diversity of *Vanilla* in the country, the apparent conservation threats, and the actions needed to develop an integrated strategy for the conservation and sustainable use of these valuable genetic resources in the country. Many of the actions we suggest will also be relevant for the conservation management of this valuable genus in other countries.

***Vanilla* diversity in Colombia**. Colombia is the most biodiverse country for the Orchidaceae, with close to 4,300 species reported, including for the genus *Vanilla* (Ministerio de Ambiente y Desarrollo Sostenible [MADS] y Universidad Nacional de Colombia [UNAL] 2015). This genus in the basal subfamily Vanilloideae comprises monopodial, hemi-epiphytic to epiphytic climbing vines with branching stems. The genus has a pan-tropical distribution, and consists of roughly 110 species (Soto-Arenas & Cribb 2010; and

Received 3 May 2016; accepted for publication 13 July 2016. First published online: 30 August 2016.

see Koch *et al.* 2013, Molineros-Hurtado *et al.* 2014, Pansarin *et al.* 2012, 2016). Within the neotropical region approximately 53 species are recognized, belonging to three distinct phylogenetic lineages: the basal membranaceous clade (*Vanilla* subgen. *Vanilla* sect. *Membranacea* Porteres) with 15 species; five Caribbean species that are phylogenetically related to African species; and finally the so-called aromatic clade (*Vanilla* subgen. *Xanata* sect. *Xanata*) with 33 species (Soto-Arenas & Cribb 2010). The cultivated species *V. planifolia*, as well as other species with aromatic fruits, fall within this clade.

The study of taxonomy and distributions of *Vanilla* has been hampered due to the rarity of plants in natural habitat, their low flowering frequency and ephemeral flowers (lasting generally no more than 24 hours). Based on a review of literature and specimens deposited in the major international herbaria, Soto-Arenas & Cribb (2010) registered eleven species for Colombia, ten of which fall in the aromatic clade. Herbaria in Colombia were, however, not consulted. Recent work in the field in Colombia has identified natural populations of twelve species, including four new reports for the country: *V. bicolor*, *V. cribbiana*, *V. helleri*, and *V. phaeantha*, and a confirmation of wild populations of *V. planifolia* (Molineros-Hurtado 2012, Molineros-Hurtado *et al*., unpubl. data). Additionally a new species, *V. rivasii* has been described, named after the campesino who found the plant (Molineros-Hurtado *et al.* 2014). Ortiz-Valdivieso (2015) listed two further species for the country, *Vanilla palmarum* and *Vanilla penicillata* both from the Amazon region. All these additional reports constitute species in the aromatic clade. The Colombian MADS & UNAL (2015) report three additional species belonging to the Membranaceous clade: *V. guianensis*, *V. inodora* and *V. mexicana*, although these reports lack associated reference specimens, and may need botanical verification. Table 1 lists the reported species for Colombia, with sources. Figure 1 illustrates a number of Colombian native *Vanilla* species in natural habitat.

These reports bring the total number of species for Colombia to 22, of which 18 fall within the aromatic clade. Three of these aromatic species, *Vanilla columbiana*, *V. espondae* and *V. rivasii* are endemic to the country. Thus, Colombia represents an important center of diversity for this genus, and in particular for the aromatic clade. The Chocó Biogeographic region on the Pacific coast of Colombia is notably diverse, with eight aromatic species. For Central America and Mexico 15 *Vanilla* species are reported (Soto-Arenas & Dressler, 2010). Soto-Arenas and Cribb (2010) recognize 26 species in Brazil, where further species have been described recently (Pansarin *et al.* 2012, Pansarin & Miranda 2016), including *Vanilla labellopapillata*, affiliated to *V. insignis* and *V. odorata* (Koch *et al*. 2013).

The natural populations of aromatic *Vanilla* species in Colombia comprise important genetic resources for vanilla crop improvement, including both wild, locally adapted populations of *V. planifolia*, as well as other aromatic species. These crop wild relatives represent the secondary gene pool of cultivated vanilla, and some have already proved of value in cultivar development. For example, the cultivated *V. x. tahitensis*, grown exclusively in Tahiti, possesses distinct, valued organoleptic properties, and has been determined to be of hybrid origin between the two parental species *V. planifolia* and *V. odorata* (Lubinsky *et al*. 2008). *Vanilla pompona* has been used to develop hybrid cultivars with *V. planifolia* in Central America with an increased resistance to *Fusarium* infection (Belanger & Havkin-Frenkel 2011).

In addition to their use in crop breeding programs for *Vanilla planifolia*, the direct cultivation of these native aromatic *Vanilla* species provides a promising opportunity for sustainable use of biological diversity, offering new products in a diversified fragrance and aroma market. Considerable differences exist in the relative compositions of volatiles both between species (Ranadive 2011), and between *V. planifolia* cultivars from different regions (Salazar-Rojas *et al.* 2011), and, as seen with *V x. tahitensis*, these differences may be of commercial interest.

In addition to consideration of plant diversity, the biological resources represented by the microbial diversity associated with wild *Vanilla* species must also be included in a comprehensive conservation program. Beneficial symbionts with positive functional roles in the natural environment may be of significant value in developing eco-efficient agricultural practices for the crop, and enhancing strategies for population re-introductions.

Table 1. Register of presence of *Vanilla* species in Colombia. Historical distributions are based on herbarium records.

***Vanilla* species**	**Current known or Historical Distribution in Colombia**	**Citation for presence in Colombia**
Vanilla bicolor Lindl.	Chocó	Molineros-Hurtado, 2012; Molineros *et al.*, unpubl. data
Vanilla calyculata Schltr.	Andean-Valle del Cauca	Molineros-Hurtado, 2012; Molineros *et al.*, unpubl. data
Vanilla columbiana Rolfe	Andean-Valle del Magdalena (historical)	Soto Arenas, 2010
Vanilla cribbiana Soto Arenas	Chocó	Molineros-Hurtado 2012; Molineros *et al.*, unpubl. data
Vanilla dressleri Soto Arenas	Chocó	Molineros-Hurtado, 2012; Molineros *et al.*, unpubl. data
Vanilla espondae Soto Arenas	Andean-Valle del Magdalena (historical)	Soto Arenas, 2010
Vanilla helleri Hawkes	Chocó	Molineros-Hurtado *et al.*, unpubl. data
Vanilla hostmanii Rolfe	Amazonia	Molineros-Hurtado *et al.*, unpubl. data
Vanilla guianensis Splitg.	Not registered	MADS & UNAL, 2015
Vanilla inodora Schiede	Not registered	MADS & UNAL, 2015
Vanilla methonica Rchb.f & Warsz.	Amazonia (historical)	Soto Arenas, 2010
Vanilla mexicana Mill.	Not registered	MADS & UNAL, 2015
Vanilla odorata C. Presl	Chocó; Andean	Molineros-Hurtado, 2012; Molineros *et al.*, unpubl. data
Vanilla palmarum Lindl	Amazonia (historical)	Ortiz-Valdivieso, 2015
Vanilla penicillata Garay & Dunst	Llanos (historical)	Ortiz-Valdivieso, 2015
Vanilla phaeantha Rchb.	Llanos; Carribean	Molineros *et al.*, unpubl. data
Vanilla planifolia Andrews	Chocó	Molineros-Hurtado 2012; Molineros *et al.*, unpubl. data
Vanilla pompona Schiede	Andean-Valle del Cauca	Molineros *et al.*, unpubl. data
Vanilla riberoi Hoehne	Amazonia (historical)	Soto Arenas, 2010
Vanilla rivasii Molineros-Hurtado et al	Chocó	Molineros-Hurtado, 2012; Molineros *et al.*, unpubl. data
Vanilla sprucei Rolfe	Amazonia (historical)	Soto Arenas, 2010
Vanilla trigonocarpa Hoehne	Chocó	Molineros-Hurtado 2012; Molineros *et al.*, unpubl. data

Threats to *Vanilla* conservation. The exceptional diversity of *Vanilla* in Colombia is subject to severe conservation threats, both due to intrinsic biological traits of these species, and to extrinsic anthropogenic activities.

Vanilla species, in common with many orchid species, are rare in natural habitats, and populations discovered in Colombia often comprise only a few, or a single individual. Flowering is rare, and natural pollination events are low (Molineros-Hurtado, 2012). *Vanilla*, like all orchids, requires an association with mycorrhizal fungi for seed germination (Porras-Alfaro & Bayman 2007). Only one or two clear germinated propagules have been seen during field work, and natural reproduction of these species most likely includes considerable vegetative propagation, due to natural vine division (Flanagan & Mosquera-Espinosa, unpub. data 2009-2015), leading to low genetic diversity in

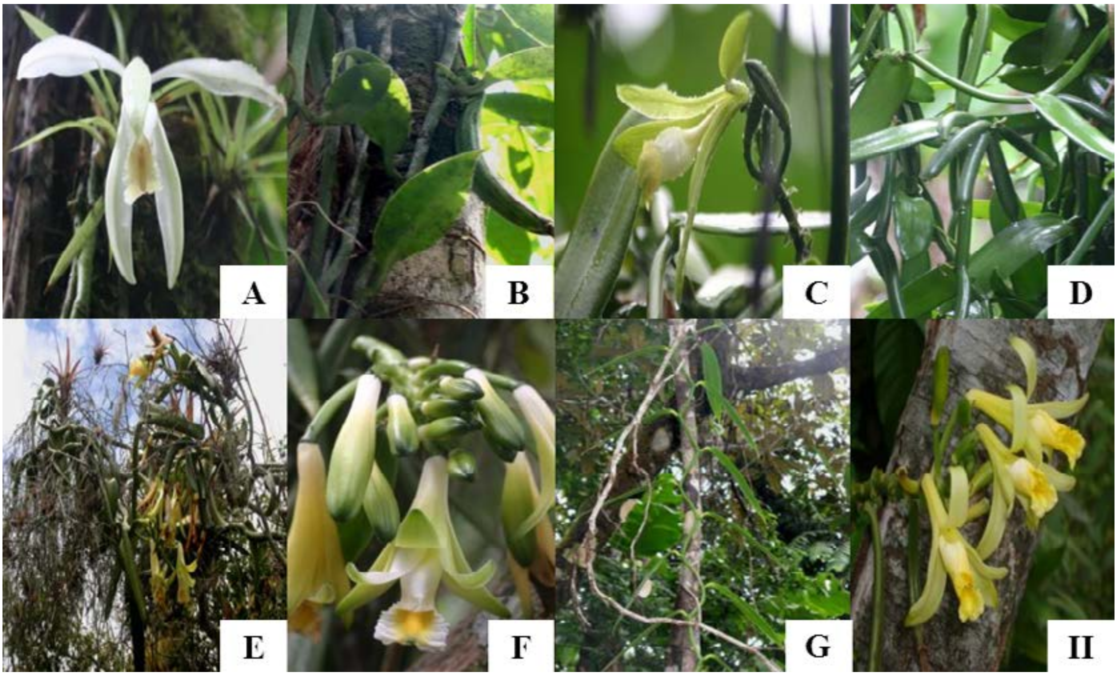


FIGURE 1. Native species of *Vanilla* in natural habitat in Colombia: *Vanilla trigonocarpa* - A. Flower, B. Developing fruit; *Vanilla phaeantha* - C. Flower D. Developing fruit; *Vanilla calyculata* - E.. habit, association with bromelias in subxerophytic shrubland. F. flower. *Vanilla rivasii* G. growth habit in tropical humid forest. H. inflorescence with three flowers opens simultaneously, and developing fruits. (Photos: A–G: Flanagan, 2009-2015; H: Molineros-Hurtado, 2011).

populations. The combination of small population sizes, and low genetic diversity from clonal reproduction makes these species extremely vulnerable to population, and eventual species extinction (Ellstran & Elam 1993).

Vanilla species have a natural lowland tropical distribution. In Colombia they associate with either the humid tropical forests along the Pacific littoral and in the Amazon region, with the dry tropical forests found in the Caribbean region, the inter-Andean valleys of the Magdalena and Cauca rivers, or in the riparian forests in the ´Llanos´ region of the Orinoco watershed in the Northeast of the country. *Vanilla* species are rarely found above 1200 masl at this latitude. Each species in Colombia tends to be limited in distribution to one or sometimes two of the five biogeographic regions: Pacific, Andean, Caribbean, Amazonia and Llanos (Table 1). Colombia contains two recognized biodiversity hotspots, The Northern Andes, and the Tumbes-Chocó-Magdalena (Myers, Mittermeier, Mittermeier, da Fonseca & Kent, 2000), and the Chocó region on the Pacific coast harbors unparalleled plant biodiversity (Gentry, 1986). All of these habitats are under considerable threat of deforestation, due to the continued direct impact of habitat conversion for agriculture and mining (both legal and illegal), as well as the increasingly common forest fires (González *et al.* 2011).

In 2014, the Colombian national deforestation rate registered an increase of 14% compared with the previous year, with a total of 140,356 Ha lost. Most deforestation is occurring in the Amazon and Andean regions. Nonetheless, the tendencies in the Caribbean and Pacific regions are the more troubling for the *Vanilla* genus. Only 4% of the original cover of tropical dry forest remains in Colombia (Pizano & García 2014), and in 2014, the Caribbean region, with only 3% forest cover, constituted 14% of the total national forest loss, with a total loss of 18.903 Ha. In the Chocó biogeographic region along the Pacific littoral, which still retains a high proportion of natural forest habitat, the deforestation rate more than doubled from 2013 to 2014 (IDEAM, 2015). In the field we have found populations of eight species associated with humid forest in the Pacific region, and three species with distributions in tropical dry forest (Table 1). For several species only a single locality is known.

In addition to habitat degradation, orchids, being of commercial interest, are subject to the pressure of collection from wild populations (Calderón-Sáenz 2007). In Colombia, *Vanilla* is currently not much sought-after compared with other ornamental orchid species. However, as interest grows in its cultivation, it is likely that vines will be increasingly extracted from the wild. With species determination being problematic due to infrequent flowering, it is probable that both commercial and non-commercial species will be targeted, as has been seen in Central America and Mexico, where fewer than 30 wild plants of *V. planifolia* are known (Schluter *et al.* 2007).

Political strategies for *Vanilla* conservation in Colombia. In 2015, a National Plan for the Study and Conservation of Colombian Orchids was published (MADS & UNAL, 2015). This document details the legal framework that exists at the national and international level for the protection of orchid species. The entire Orchidaceae family, with some exceptions, is listed in Appendix II of the CITES Convention as species that may become threatened with extinction if trade is not closely controlled. A handful of species are listed in Appendix I as threatened with extinction and requiring further protective measures. It is important to note that Appendix II of CITES exempts the "fruits, and parts and derivatives thereof, of naturalized or artificially propagated plants of the genus *Vanilla"*. Equally, seeds, seedpods, and pollen (including pollinia), and tissue cultures obtained *in vitro* are also exempt.

In 2007, the Red list for Orchid species in Colombia was published (Calderón-Sáenz 2007). This volume details 375 species across the more threatened genera, with more than half being categorized within the higher risk categories of Critical (CR), Endangered (EN) or vulnerable (VU). The genus *Vanilla* was not assessed, and the lack of population data for these species hinders a comprehensive threat assessment. To date, only a small number of small populations are known, and it is probable that most *Vanilla* species in Colombia fall within a higher category of risk.

The National Plan for the Study and Conservation of Colombian Orchids aims to have all Colombian orchid species prioritized for conservation by 2025, with Colombia recognized as a leading country for orchid conservation. Given the small, infrequent populations of *Vanilla* we have found in the wild, it is of considerable concern that the entire genus of *Vanilla* is excluded from CITES Appendix II. In particular, exempting "naturalized or artificially propagated plants of the genus *Vanilla*" from the Appendix II may make enforcement of wildlife regulations more difficult, as a wild-sourced plant can easily be presented as artificially propagated. With increased attention on *Vanilla* wild populations for commercial ventures, it may be necessary to clarify this exemption, limiting it to only the major cultivated species of *V. planifolia, V. pompona*, and *V. x. tahitensis*, with plants being exempt only if they are of certifiable artificial propagation. To improve protection for populations of *Vanilla* species native to Colombia, it is also important to assess these species for listing in the IUCN Red List of Threatened Species. Currently, not one species from the aromatic clade of *Vanilla* is listed in the IUCN. These measures will be an important step forward to achieving in Colombia, as in Mexico under the government resolution NOM-059-SEMARNAT-2010, a special protection for *Vanilla* biodiversity.

The Colombian National Plan details a number of actions necessary for effective conservation of the orchid diversity of the country. These measures range from continued study of taxonomy, diversity, physiology and ecology of native orchid species, establishing priorities for conservation action, including the *in situ* conservation of orchid populations and their habitats, the development of sustainable strategies for commercialization, and promoting awareness regarding the conservation needs in local communities.

In the face of threats from habitat degradation, and uncontrolled exploitation of wild populations, there is an urgent need for effective integrated strategies for orchid conservation. In the case of *Vanilla* in Colombia, these actions must be swift, to take effect before limited populations disappear from the wild. We here outline the necessary conservation actions, including *in situ, ex situ* and *circa situm* measures, and their application in the context of *Vanilla* conservation in Colombia. Table 2 provides an overview of these actions.

TABLE 2. Activities for an integrated strategy for conservation and sustainable use of native *Vanilla* species in Colombia.

Political strategies
Evaluate the status of the whole of the genus *Vanilla* as exempt from CITES Appendix II.
Evaluate all species of *Vanilla* at the national level for inclusion in the IUCN Red List of Threatened Species.
Consider special National protection for native *Vanilla* species.
Develop strategies to enforce existing national and international conservation legislation, including forensic genetic tools.
Certification of authorized sustainable sources of *Vanilla* germplasm for commercialization.
***In situ* conservation actions**
Gap Analysis to ensure inclusion of *in situ Vanilla* populations in protected area networks, as well as maximum coverage of genetic diversity in *ex situ* collections.
Research program on biogeography, genetic diversity, ecology, phytochemistry and physiology of *Vanilla* species in natural habitat.
Development of DNA Barcodes for native *Vanilla* species, and further genetic profiling for forensic applications.
Develop a strategy for population restoration, including population augmentation, and reintroductions, both within and beyond natural ranges.
***Ex situ* conservation actions**
Establishment of an in-country living collection of representative genotypes, with at least two duplications.
Establishment of a cryopreserved seed bank, and associated research program to develop optimal long-term storage protocols, including synthetic seeds.
Establishment of *in vitro* germplasm collection, maximizing genetic diversity, and an associated research program to develop efficient protocols for *ex vitro* acclimatization for cultivation and population restoration.
Large-scale *in vitro* multiplication of certified planting material for prioritized genotypes.
Establishment of an orchid mycorrhizal and endophyte germplasm collection, and associated research into functional application of microbial symbionts in *ex situ* propagation, and population restoration & cultivation.
***Circa situm* conservation actions and sustainable use**
Evaluation of market possibilities for diversified vanilla products from distinct species, and for organic or other product certification.
Establishment of community-based *circa situm* cultivation initiatives, including community germplasm banks as living collections.
Characterization of native *Vanilla* genetic resources for traits of agronomic and market interest, including phytosanitary evaluation of plant-microbe interactions.
Capacity building for good agro-ecological practice for vanilla cultivation, pollination and post-harvest processing.

***In situ* measures for *Vanilla* conservation**. Protection of the natural habitat – both actual, and that predicted for future distributions under climate change scenarios – is the single most important conservation action for most plant species, although additional measures are also needed to ensure the survival of populations in natural habitat. *In situ* conservation facilitates the maintenance of a sufficient population size to retain genetic variation and viability. Additionally, it enables conservation of the ecological interactions in which the species are involved, thus also contributing to the conservation of other species in inter-dependent ecological networks. Moreover, *in situ* conservation permits ongoing adaptive change in response to environmental variation, thus maximizing persistence of the species in the long-term (Maxted, Ford-Lloyd & Hawkes, 1997). To achieve effective *in situ* conservation the following actions should be taken.

Continued research on Vanilla *species in their natural environments —*. As the National Plan for the Study and Conservation of Orchids (MADS & UNAL, 2015) makes abundantly clear, for the vast majority of Colombian orchids, including *Vanilla* species, much more information is needed to develop effective conservation strategies. A broad research program is required that includes taxonomical delimitations, population distributions (both current and potential under climate change scenarios), and ecological characterization of natural habitats. In addition, studies on population biology, genetic diversity both within and between populations, and ecological interactions, in particular with pollinators, mycorrhizal fungi and seed dispersers, are needed to better understand requirements for population persistence. Furthermore, with a view to promote sustainable cultivation systems, studies aimed at understanding plant physiology under both natural and agro-ecological conditions, and for the characterization of organoleptic traits are required.

Establish distributions and determine representation in protected areas —. In order to maximize the protection given to natural plant populations it is important that their natural distributions are included within the network of *in situ* protected areas. In Colombia this network comprises both public and private protected areas. Our studies have found a concentration of *Vanilla* diversity in the Chocó biogeographic region. This region retains 50% of native forest cover (Gillespie *et al*. 2012), yet less than 5% of this area is included in the Protected Area Network (Forero-Medina & Joppa 2010). Most of this region is covered by the autonomous territories of the indigenous and afro-descendent communities, making participative conservation initiatives with these communities key to the success of an integrated strategy for *Vanilla* in Colombia, as discussed below (WWF Colombia 2008).

A thorough gap analysis for the genus in the country is advisable in order to identify areas where *in situ* conservation measures should be prioritized, as well as to ensure that the maximum amount of genetic diversity in the country is represented in *ex situ* collections (Maxted, Dulloo *et al.* 2008). In Colombia, a single model of species distribution has been developed for *Vanilla odorata*, but this is limited to only the Department of the Valle del Cauca (Reina-Rodriguez 2011). Given the broad distributions of many *Vanilla* species, this is an action that must be conducted at the national level, or above, and for all species in the genus. Our work has mainly focused on the Pacific and Andean regions of Colombia, and continued botanical studies are urgently needed in the Amazon, Llanos and Caribbean regions.

Ecological and physiological studies —. Ecological studies in *Vanilla* are limited by the scarcity of natural populations for study, as well as the ephemeral flowering, and pollination visits. Pollination in many aromatic species is likely undertaken by male Euglossine bees (Soto-Arenas 2003, Pansarin 2016). Within the aromatic clade, Roubik and Ackerman (1987) observed bees in the genera *Euglossa*, *Eulaema* and *Eufresia* with pollen from *V. planifolia*, *V. pompona*, and other unidentified species. Pollination of *V. trigonocarpa* by *Euglossa asarophora* is reported (Soto-Arenas & Dressler 2010), and the aromatic compound vanillin strongly attracts *Eulaema cingulata* (van der Cingel 2001). There is evidence of self-pollination in *V. palmarum* and *V. bicolor* (Householder *et al*. 2010; Van Dam *et al*. 2010), and some spontaneous self-pollination may occur in *V. planifolia* (Soto-Arenas 2003). In Cuba, where no Euglossine bees are present (Genaro 2008), naturalized populations *of Vanilla planifolia* present natural pollination events. These fruiting events may be the result of visits by *Melipona beecheii* Bennett, 1831, or from self-pollination (Angel Vale, pers. comm.). The membranaceous species, *Vanilla edwallii* is pollinated by males bees in the genus *Epicharis* (Apidae: Centridini) (Pansarin *et al*. 2014).

As effective pollination is needed to maintain population viability in the wild, it is vital to understand pollination biology, and take measures to also ensure the conservation of pollinator species. Furthermore, the presence of self-pollination is an agronomic trait of considerable interest, with the potential to greatly reduce the labor needed for this hand-pollinated crop. Pollination studies of native species in Colombia are lacking.

Vanilla, like all orchid species, relies on a mycorrhizal interaction with a fungal symbiont for seed germination, and subsequent plant growth, nutrition and defense. Research into this area is discussed in the *ex situ* section below. Physiological studies are also recommended to characterize traits of potential agronomic and commercial interest, as well as the performance of interspecific hybrids of interest. These traits should include studies of phytochemistry or organoleptic traits in order to assess market opportunities. For both conservation and cultivation, environmental characterization of habitats is also necessary for planning translocation activities.

Genetic diversity studies —. In order to implement effective conservation management and sustainable use strategies, an understanding of the structure of the genetic diversity both within and between species is recommended. While phylogenetic relationships are discernable between aromatic species using a traditional DNA sequence loci (Lubinsky *et al.* 2008, Soto-Arenas & Dressler 2010, Molineros-Hurtado *et al*. unpubl. data), intraspecific genetic structure is less clear, with low levels of genetic variation detected within species (e.g. Schluter *et al.* 2007). However, new techniques in genotyping (Narum *et al.* 2013) offer the potential for higher resolution genetic characterization for ecological and agronomic profiling. Ideally this should be conducted across species distributions, and at the supranational level where possible.

Genetic characterization also offers effective tools for the enforcement of environmental legislation, including CITES. An important step forward in this area is the development of the DNA Barcodes for the *Vanilla* species native to Colombia, thus providing a necessary tool for the identification of species from vegetative material (González & Paz 2013). A more detailed genotype dataset from next generation sequencing methods would also allow for the determination of population of origin of illegally collected material.

Population restoration —. Due to the naturally small and rare populations of native *Vanilla* species, conservation translocation actions are advisable to maintain species in the wild. In particular translocation is recommended to more secure protected areas, including members of the network of private reserves, which often have a more restricted access than the National Parks system (Asociación Red Colombiana De Reservas Naturales De La Sociedad Civil; Resnatur.org.co).

Translocation activities may be one of four different actions. Population augmentation involves the addition of plants to an already existing population. Plants may also be reintroduced to a site where the species was previously recorded, or to a site within the species historical range, but having no previous record of presence (within range introduction). Finally, a new population may be established with conservation aims to a site beyond the recorded historical range (Liu *et al.* 2015). There is a particular need to apply this latter measure in the face of impact of climate change on plant species distributions.

In Colombia, we have successfully conducted population augmentation actions for *Vanilla calyculata* using material sourced from the same location and vegetatively propagated before re-introduction (Flanagan *et al*. 2012). However, further translocation actions for all aromatic species, including within range and beyond range introductions to establish populations in protected areas, should be considered. Such translocations would also encompass the objective of establishing *ex situ* living collections (see below). In planning conservation translocation actions, a prior study of genetic diversity is recommended to help determine appropriate sources for plant material. The application of asymbiotic or symbiotic *in vitro* propagation from seed (see *ex situ* below) offers a promising avenue to propagate material for translocation while also promoting genetic diversity.

In *in situ* conservation activities, it is important that the demographic parameters of both translocated populations and natural populations should be monitored periodically to ensure ongoing population viability (Tremblay & Hutchings, 2003).

***Ex situ* measures for *Vanilla* conservation**. Although managed *in situ* measures represent the ideal for plant conservation, for orchids this strategy faces the challenge of the copious, uncontrolled extraction of plants from natural habitat. Thus, although the designation of protected areas and population monitoring are essential, they are not sufficient actions by themselves for orchid protection. *Ex situ* approaches must be implemented in a complementary manner to ensure species conservation, as also indicated in Article 9 of the Convention for Biological Diversity (Hagsater *et al.* 1996, Seaton *et al.* 2010, 2013). Furthermore, *in situ* activities such as reintroduction into natural habitats may require initial *ex situ* conservation actions, with *in vitro* propagation of planting material. An integrated conservation strategy must implement all of these actions for each species in order to maximize the preservation of *Vanilla* genetic diversity.

Ex situ conservation strategies involve the conservation of a species´ genetic diversity outside of the natural habitat, through the establishment of living collections, in botanical gardens or universities, and also the preservation of genetic diversity in seed or *in vitro* tissue banks (Seaton & Pritchard 2003). In the case of orchids, *ex situ* conservation must also incorporate compatible mycorrhizal fungi (Swarts & Dixon 2009a), thus enhancing the efficacy of future population restoration activities.

Living collections —. Plants growing in secure *ex situ* conditions are an important complementary action in an integrated strategy for *Vanilla* conservation. However, the demands of space, particularly for *Vanilla* plants, limit the number of individuals, or distinct genotypes that can be maintained, thus limiting the representative genetic diversity conserved. Nonetheless, living collections are a key resource for research purposes, as well as being essential for new cultivar development. This is particularly important for *Vanilla* given the several years' time lapse from *in vitro* propagation or germination to flowering. If a sufficient, albeit limited number of living plant accessions can be maintained, these can provide the material for developing methodologies for long-term germplasm conservation in cryopreservation, as well as for studies of agronomic traits, phytochemistry and plant physiology and ecology, in particular plant-microbe interactions (see, for example, Koyyappurath *et al.* 2015b). The PVBMT Research Unit of CIRAD on the Island of Reunion maintains a collection of 400 living vanilla genotypes, from over 23 species, of which ten are from the aromatic clade (Roux-Cuvelier & Grisoni 2010). Several further small collections exist outside of the natural distribution of the aromatic clade. However it is vital that *ex situ* conservation and research collections be established in the country of origin of the plant material.

Ideally, living collections should be replicated in-country, in at least two localities, between partner institutions, either universities, or botanical gardens, and ideally form part of a global conservation strategy for the genus. Orchid collections do exist in some of the larger botanical gardens in Colombia, yet these are mainly located beyond the natural altitudinal range for *Vanilla*. Species registered as present in Colombian collections are *V. calyculata*, *V. pompona*, *V. planifolia* and *V. spp*. The Jardín Botánico de Tulúa "Juan María Céspedes", in the Cauca River Valley, at approximately 1000 m.a.s.l., contains three of these species (MADS & UNAL 2015).

For *Vanilla*, as for many orchids, a further possibility is to work together with local communities within the native distributions to develop *circa situm* community germplasm banks. This is discussed further below.

Seed banks and other cryopreservation techniques —. Seed conservation has been a central tenet for conservation of genetic diversity in crop plants for several decades, and for the Orchidaceae seed conservation initiatives are growing, in particular the Orchid Seed Bank Challenge in Western Australia (Swarts & Dixon 2009a, 2009b) and the global initiative Orchid Seed Stores for Sustainable Use (OSSSU) (Seaton 2007, Seaton *et al.* 2010, Seaton & Pritchard 2011).

Research suggests that many orchid seeds stored at low humidity and low to ultra-low temperature may maintain viability over several decades. Given the small size of seeds, this method can effectively store a good representation of genetic diversity of each species in a small space (Merritt *et al.* 2014, Seaton *et al.* 2010). Nonetheless, some seeds have much lower viabilities, even at low temperature, and there is an urgent need for more research on the physiology of orchid seed storage and regeneration, in order to develop optimal long-term storage and regeneration conditions for different species (Divakaran *et al.* 2006, Merritt *et al.* 2014, Popova *et al.* 2016;).

Little attention has been paid to *Vanilla* seed storage. *Vanilla* species, with their hard, waxy seed testa adapted for withstanding passage through vertebrate intestinal tracts, are most easily germinated from immature fruits (Menchaca *et al.* 2011). However, storage of immature seeds under cryopreservation may not be effective. The application of innovative cryopreservation techniques including seed encapsulation-dehydration and vitrification of both seeds and other material such as pollen, *in vitro* meristematic tissue, or protocorms needs to be more widely explored (Merritt *et al.* 2014, Popova *et al.* 2016). A comparison of cryopreservation techniques in *Vanilla planifolia* found droplet vitrification of apical material to be the most successful, although survival (30%) and regeneration (10%) success were still low (González-Arnao *et al.* 2009). Divakaran *et al.* (2006) encapsulated *in vitro* regenerated shoot buds and protocorms (3–5 mm) in 4% sodium alginate, and found these ´synthetic seeds´ could be stored successfully for up to 24 months in sterile water at 22ºC. Seed conservation is clearly a priority area for *Vanilla ex situ* conservation research, in particular exploring the use of these established protocols in the wild relatives of cultivated *Vanilla*.

In vitro *germplasm collection* —. As a complement to the maintenance of a living collection and seed banking, an *in vitro* germplasm bank is also recommended. Considerable research has been devoted to *in vitro* propagation of orchids, including *Vanilla*, both from seed and meristematic tissue. *In vitro* propagation from seed generally applies asymbiotic techniques using a complete tissue culture medium, commonly Knudson C or half-strength Murashige and Skoog (MS), and is widely used for massive multiplication of orchids for commercialization, private collections, or, less frequently, population re-introductions. An alternative promising area of research is symbiotic seed germination in association with known mycorrhizal fungal symbionts. This is discussed in the following section.

A number of research groups have developed protocols for *in vitro* propagation of *Vanilla planifolia* from meristematic tissue (e.g. Divakaran *et al.* 2006; Tan*et al.* 2010). For the efficient maintenance of an *in vitro* germplasm bank, techniques for cultivation under slow growth conditions are necessary. An *in vitro* collection of genotypes has a much smaller space requirement than an *ex vitro* living plant collection. However, *in vitro* plantlets are not subject to the physiological pressures present in the natural environment, and research is needed to ensure adequate acclimatization of plants on transfer to *ex vitro* conditions. The application of mycorrhizal fungi to enhance nutrition and plant defenses upon extraction from *in vitro* conditions offers a promising line of research (Zettler *et al.* 2007, and see below).

Once established, meristematic tissue from *in vitro* culture can be used as material for encapsulation protocols for cryopreservation, as mentioned above (Popova *et al.* 2016, Divakaran *et al.* 2006). *In vitro* propagation also offers an efficient way to propagate selected material for both commercial cultivation and reintroduction. This is particularly important in Colombia, where the early availability of high-quality *in vitro* certified planting stock before large commercial ventures are initiated, may help to minimize the pressures on natural populations.

Conservation and applications of mycorrhizal fungi and other endophytes —. *Vanilla* species, like all orchids, have a specific association with mycorrhizal fungi, which are required for seed germination and plant growth (Bayman, Mosquera-Espinosa & Porras-Alfaro 2011, Porras-Alfaro & Bayman 2007). Orchid mycorrhizal fungi and other endophytes likely also play a role in plant defense, either directly through antagonistic interactions, or indirectly through improved nutrition (Mosquera-Espinosa, Bayman & Otero 2010; Otero, Mosquera-Espinosa & Flanagan 2013).

A study of *Vanilla* mycorrhizal fungi in Puerto Rico and Costa Rica isolated three sexual fungal genera, *Thanatophorus, Ceratobasidium* and *Tulasnella* in the order Cantharellales, from both wild and cultivated adults plants of *V. planifolia*, and two other species (Porras-Alfaro & Bayman 2007). However, only isolates of *Ceratobasidium* proved effective for promoting *V. planifolia* seed germination, supporting the hypothesis that orchid mycorrhizal associations may change during plant growth and development. Further, *Ceratobasidium* fungi isolated from another orchid species, *Ionopsis utricularioides* were even more effective at inducing *Vanilla* seed germination. These cross-species *Ceratobasidium* isolates were also most efficient at promoting growth and survival of *in vitro* plantlets of a commercial cultivar. In Colombia, both *Ceratobasidium* and *Tulasnella spp*. have been isolated from adult plants of several *Vanilla* species distributed across dry and humid tropical forest habitats (Mosquera-Espinosa *et al.* 2010, 2013; Mosquera-Espinosa *et al.*, unpubl. data), revealing a consistent relationship between adult plants of *Vanilla* species and these fungal genera. However, further research is required regarding mycorrhizal fungal function for *in vitro* seed germination and plantlet growth. For this, the use of *in situ* seed baits offers a promising technique to isolate those fungi with which the seeds associate for germination under natural conditions (Brundrett *et al.* 2003, Zettler *et al.* 2011).

Effective regeneration of plants from *ex situ* germplasm storage requires that these essential fungal symbionts are also conserved. Cryopreservation techniques have been developed to store mycorrhizal fungi either together with orchid seed (e.g. Wood *et al.* 2000), or separately (Batty *et al.* 2001, Ercole *et al.* 2013). Isolates of *Tulasnella* and *Ceratobasidium* genera stored at -80°C for up to 24 months retained or even improved their ability to promote *in vitro* seed germination (Ercole *et al.* 2013). It is essential that *ex situ* germplasm storage of mycorrhizae be implemented alongside *Vanilla* seed storage to ensure the success of long-term *ex situ* conservation initiatives.

While most attention has been paid to mycorrhizal fungi, other endophytes, both fungal and bacterial, may play beneficial roles in *Vanilla* ecology and cultivation (Porras-Alfaro & Bayman 2011). Endophytic fungi, including those involved in mycorrhizal associations are promising antagonistic agents for crop pathogen control, reducing the use of agrochemicals and making production systems more eco-efficient (eg. Mosquera-Espinosa *et al.* 2013). In a study of endophytic fungi of five *Vanilla* species in Colombia, we found a high diversity of fungi, with 148 Operational Taxonomic Species (OTUs) belonging to eleven Ascomycete and four Basiomycete orders (Mosquera-Espinosa *et al.* Unpubl. data). The evaluation of functional traits of this microbial diversity is now required. The bacterium, *Bacillus amyloliquefaciens*, which has *in vivo* antifungal properties in other plant species, has been isolated from *V. planifolia* tissues (White *et al.* 2014). In a study of endophyte communities of fruits in cultivated vanilla, Khoyratty *et al.* (2015) concluded that fungal endophytes may play an important role in the development of organoleptic properties.

The study of the ecology and physiology of endophyte communities in *Vanilla* is an incipient, yet significant area of research. The diversity of wild populations known in Colombia offers a valuable opportunity to study naturally occurring endophytic communities, and at the Pontificia Universidad Javeriana in Cali current research activities are focused on developing an effective *ex situ* conservation strategy for the *Vanilla* Endophyte Collection, as well as evaluating these valuable microbial resources for beneficial actions in the conservation, sustainable use and phytosanitary protection of the diversity of native *Vanilla* species in Colombia.

***Circa situm* conservation and sustainable use**. In Colombia, indigenous and afro-descendent communities living in rural areas have autonomous jurisdiction over their traditional territories. The majority of the Pacific Chocó region falls within these collective jurisdictions, which also include autonomous determination for the use of the biological resources within the territories. Thus, in the Chocó region, and other areas with collective territories, any actions for the conservation and sustainable use of the native vanilla resources must be carried out in a participative manner with local communities.

Given the limitations of both *in situ* and *ex situ* approaches, and the autonomy of these rural communities, a *circa situm* conservation system may offer a further complementary conservation strategy. *Circa situm* conservation refers to conservation actions within altered agricultural landscapes (e.g., agroforestry systems or home gardens) outside natural habitats but within a species' native geographical range (Dawson *et al.* 2013).

In Colombia we have worked with the community of Atuncela, a locality within the range of *Vanilla calyculata* in the Western Andean Cordillera to develop a community germplasm collection (Flanagan *et al.* 2012; Mosquera-Espinosa *et al.* 2012). This has involved the documentation of the different populations within the locality, and a planned strategy for *ex situ* vegetative propagation. Within-range population augmentation in remaining native forest fragments was also conducted. It is important that this initiative is now repeated in other localities for other *Vanilla* species native to Colombia; however such activities have a high investment of time due to the need for a close accompaniment for the community members.

Although there is no tradition of vanilla cultivation in the country, the market value of the crop offers these often forest-dependent communities a potential income for sustainable livelihood improvement. *Vanilla* plants can be easily incorporated into agroforestry systems, whether in humid tropical forest with the species such as *V. planifolia* or *V. rivasii* (Molineros-Hurtado 2012), or tropical dry forest with species such as *V. calyculata* (Flanagan *et al.* 2012).

However, given the fluctuating global price for vanilla, a more constant income may be ensured through the implementation of market product diversification, including the commercialization of fruits from different species with different organoleptic properties, as well as considering certification for organic or sustainable agricultural production, or ′biodiversity-friendly′ cultivation. These commercial possibilities should be evaluated from the outset. Additionally, increasing awareness within the national market of the advantages of natural vanilla compared with the synthetic vanillin flavoring, could promote demand at the national level, rather than depending on the international market. The high initial investment, and a slow return can make establishing community-based cultivation initiatives a big challenge (Soto-Arenas 2006), although it offers many advantages for conservation programs.

Community-based cultivation initiatives promote appreciation amongst the community of the value of the *Vanilla* resources in their territories, thereby encouraging community conservation actions for *Vanilla*, as well as promoting the more biodiverse agro-forestry ecosystems in which the vanilla is cultivated. *Circa situm* conservation through sustainable cultivation also minimizes the loss of beneficial ecological interactions, including those with pollinators and microorganisms. Furthermore, the generation of an additional income source may alleviate current pressures on communities to resort to unsustainable means for income generation, such as timber extraction, or land clearance for cattle grazing.

The challenges faced in implementing programs for sustainable production and on-farm conservation include the need for technical training and accompaniment in effective eco-agricultural practice, for at least the duration of the initial crop establishment period of two to three years. Training is also required in manual pollination to augment production, and in the post-harvest curing process. With no tradition of vanilla cultivation in Colombia, the establishment of a regional network together with other community-based producers in countries such as Mexico could facilitate the capacity-building for vanilla cultivation in Colombia.

Research on plant-microbe interactions for eco-efficient cultivation —. Worldwide, commercial production of vanilla is severely affected by phytosanitary problems, in particular root and stem rot caused by the fungal pathogen, *Fusarium oxysporum* f. sp. *radicis-vanillae* (Koyyappurath *et al.* 2015a) with a high specificity and pathogenicity to vanilla cultivars (Pinaria, Liew & Burgess, 2010). Once infested, the elimination of this fungus from cultivation soils is extremely difficult (Hernández-Hernández, 2011). Recent studies have shed light on the physiological basis for resistance to this pathogen, highlighting accessions with resistance traits (Koyyappurath *et al.* 2015b). However research into plant-microbe interactions in wild populations of *Vanilla* may facilitate the development of further strategies for disease control, as well as understanding of processes of adaptation to new agroecological conditions, including under predicted climate change scenarios.

Endophytes, including mycorrhizal fungi are promising antagonistic agents for pathogen control

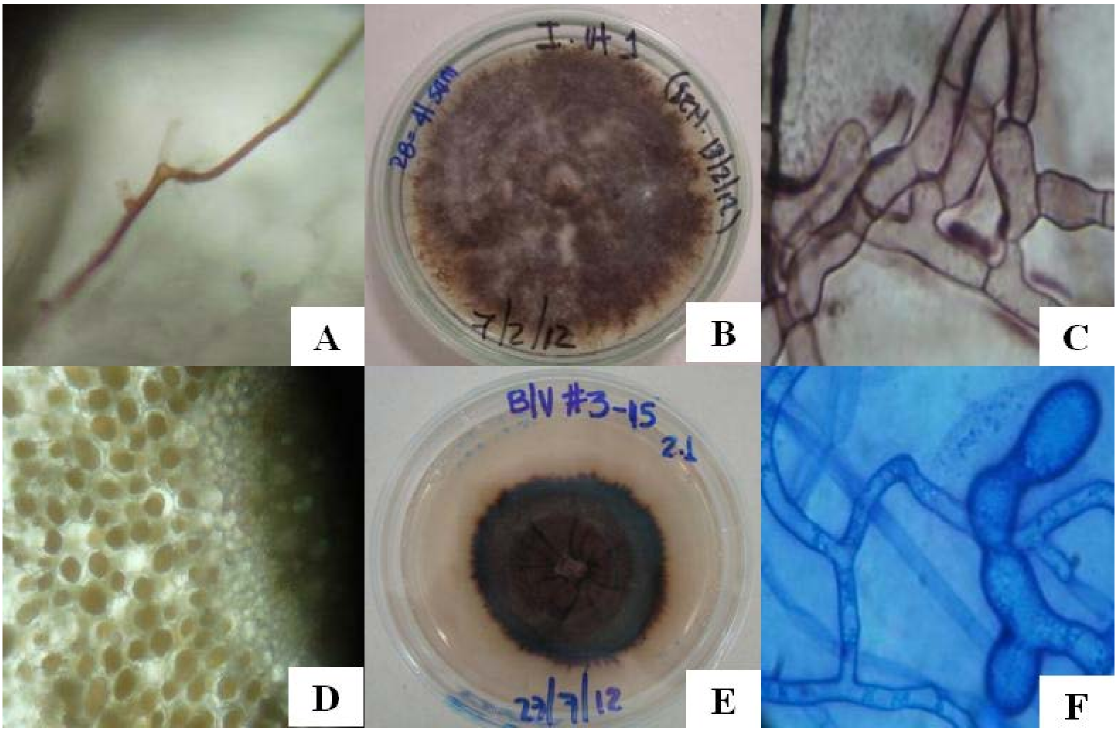


Figure 2. Dark Septate Endophytes (DSEs) and Orchid Mycorrhizal Fungi (OMF) present in host roots. A. DSE hyphae in rhizoplane 40X. B. Dark pure culture of DSE. C. DSE Septate hyphae 40X. D. OMF hyphae forming peloton in cortex 40X. E. Dark pure culture of OMF. F. OMF Septate hyphae, showing right angles and monilioid cells 40X. (Photos: Mosquera-Espinosa, 2012-2015).

and may also act to induce pathogen resistance in the plant (Bayman *et al.* 2011). Mosquera-Espinosa *et al.* (2013) obtained isolates of *Ceratobasidium spp.* from mycorrhizal pelotons present in roots of a number of orchid species in Colombia. When these were inoculated onto experimental rice plants that were then infected with the soil pathogen *Rhizoctonia solani*, the plants showed a significantly reduced expression of pathogenicity, indicating that the *Ceratobasidium spp.* can induce pathogen resistance in the plant host.

Other endophytic fungi that colonize roots in either an inter- or intra-cellular manner likely have functions related to the transfer of nutrients and disease resistance. A fungal group in which such functions have been documented include the so-called ´Dark-Septate Endophytes´ (DSE). These fungi in the phylum Ascomycota, characterized by melanized, septate hyphae, are almost ubiquitous within plants, and are often found associated with mycorrhizal fungi, pathogens and other endophytes (Jumpponen & Trappe 1998, Porras-Alfaro & Bayman 2011). From morphological characters, DSEs can be confused with mycorrhizal fungi. DSEs are known to be highly efficient in decomposition of organic material, and may pass these nutritional resources to a host plant in an endophytic interaction, thus stimulating host growth and disease resistance (Caldwell *et al.* 2000). Fungi in this group have been found to be associated with wild plants of native *Vanilla* species in the Colombian pacific region (Mosquera-Espinosa, unpubl. data) (Fig. 2). While research has been conducted on the diversity and seed germination function of *Vanilla* mycorrhizal fungi, there is clearly much more to be understood regarding the role of orchid mycorrhizal fungi and other endophytes, both fungal and bacterial, in both disease resistance and plant nutrition (Mosquera-Espinosa *et al.* 2012, Porras-Alfaro & Bayman 2011). In Colombia, in small scale, incipient vanilla cultivation systems, endemic plant disease has not yet proved to be a problem. In our studies of endophytic fungal communities in natural populations we have not detected the presence of *Fusarium oxysporum* f. sp. *radicis-vanillae*, although *Fusarium solani* has been identified (Mosquera-Espinosa *et al.* unpubl. data). It is important to attempt to maintain the absence of the major pathogens from these vanilla systems through the implementation of good agricultural practices, taking into consideration specific aspects of the plant material used, and the local environmental conditions.

In order to successfully establish sustainable community-based cultivation systems avoiding the negative cycles of pathogen infestation and increased agrochemical use, a research program in *Vanilla* agro-ecology is necessary. Agronomic characterization is required at the inter and intra-specific level for traits relating to organoleptic properties, crop yield, the physiological processes involved in flowering and pollination, including self-pollination, as well as functional traits related to plant performance with respect to biotic and abiotic stressors. These studies are also of relevance to vanilla cultivation systems worldwide.

The high diversity of wild *Vanilla* species in Colombia offers an excellent opportunity to understand *Vanilla* ecology and physiology under natural conditions. However it is essential that a program of agronomic research goes hand in hand with an effective strategy for conservation, integrating across *in situ*, *ex situ*, and *circa situm* measures.

Conclusion. Colombia is moving forward with biodiversity conservation strategies, and a growing community of researchers, conservation professionals, cultivators and hobbyists are dedicated to promoting orchid conservation. Within the Orchidaceae, the diversity and ecology of *Vanilla* has historically been under-researched, despite its economic importance. In Colombia, the Chocó biogeographic region, with the highest number of reported species for the country, is the region with the lowest coverage of protected areas. Given the increasing deforestation rates, in addition to a growing awareness of the economic potential of *Vanilla* species in the country, and thus increased pressure for unsustainable extraction from natural habitats, we suggest that *Vanilla* should receive priority action for conservation, and be considered a flagship species for the conservation and sustainable use of orchid diversity in the country, and in the Chocó region in particular. Effective preservation of the diversity of *Vanilla* in the country requires an integrated strategy for conservation, developing in concert measures for *in situ*, *ex situ* and *circa situm* conservation, and working together with local communities to develop participative programs for conservation and sustainable use. In the Pontificia Universidad Javeriana in Cali, together with institutional and community partners at the national and regional level, we have initiated a program of actions for research and outreach for both the conservation and the sustainable use of the *Vanilla* diversity of Colombia.

ACKNOWLEDGMENTS. The authors thank the Research Office of the Pontificia Universidad Javeriana, Cali for financial support for the Vanilla Research Program for Conservation and Sustainable Use. We also thank Don Alvaro Rivas from the community of Zacharias, Buenaventura, Valle del Cauca and Don Norman Castro, from the community of Atuncela, Dagua, Valle del Cauca for their enthusiasm for *Vanilla* conservation and sustainable use. We thank Francisco Molineros-Hurtado for permission to use his photograph of *Vanilla rivasii*.

LITERATURE CITED

Batty, A.L., Dixon, K.W., Brundrett, M. & Sivasithamparam, K. (2001). Long-term storage of mycorrhizal fungi and seed as a tool for the conservation of endangered Western Australian terrestrial orchids. *Australian Journal of Botany*, 49, 619–28.

Bayman, P., Mosquera-Espinosa A.T. & Porras-Alfaro, A. (2011). Mycorrhizal Relationships of *Vanilla* and Prospects for Biocontrol of Root Rots. In: Handbook of *Vanilla* Science and Technology (pp. 266-279), First Edition. Edited by Daphna Havkin-Frenkel and Faith C. Belanger.

Belanger, F.C. & Havkin-Frenkel, D. (2011). Molecular Analysis of a *Vanilla* hybrid cultivated in Costa Rica. In: Handbook of *Vanilla* Science and Technology (pp. 256-265), First Edition. Edited by Daphna Havkin-Frenkel and Faith C. Belanger.

Bory, S., Lubinsky, P., Risterucci, A. M., Noyer, J. L., Grisoni M., Duval M. F. & Besse, P. (2008). Patterns of introduction and diversification of *Vanilla planifolia* (Orchidaceae) in Reunion Island (Indian Ocean). *American Journal of Botany*, 95(7), 805-815.

Brundrett, M., Scade, A., Batty, A.L., Dixon K.W. & Sivasithamparam, K. (2003). Development of *in situ* and *ex situ* seed baiting techniques to detect mycorrhizal fungi from terrestrial orchid habitats. *Mycological Research*, 107, 1210-1220. doi:10.1017/S0953756203008463.

Calderón-Sáenz, E. (ed). (2007). Libro Rojo de Plantas de Colombia. In: Volumen 6. Orquídeas primer parte. Instituto Alexander von Humboldt—Ministerio de Ambiente, Vivienda y Desarrollo Territorial. Bogotá. 828 p.

Caldwell, B.A., Jumpponen, A. & Trappe, J.M. (2000). Utilization of major detrital substrates by dark-septate, root endophytes. *Mycologia,* 92, 230-32.

Dawson, I.K., Guariguata, M.R., Loo, J., Weber, J.C., Lengkeek, A., Bush, D., Cornelius, J., Guarino, L., Kindt, R., Orwa, C., Russell, J. & Jamnadass, R. (2013). What is the relevance of smallholders' agroforestry systems for conserving tropical tree species and genetic diversity in *circa situm*, *in situ* and *ex situ* settings? A review. *Biodiversity and Conservation*, 22 (2), 301-324.

Divakaran, M., Nirmal, B.K. & Peter, K.V. (2006). Conservation of *Vanilla* species *in vitro*. *Science Horticultural*, 1109, 175–180.

Ellstrand, N.C. & Elam, D. R. (1993). Population Genetic Consequences of Small Population Size: Implications for Plant Conservation. *Annual Review of Ecology and Systematics*, 24, 217-242.

Ercole, E., Rodda, M., Molinatti, M., Voyron, S., Perotto, S. & Girlanda, M. (2013). Cryopreservation of orchid mycorrhizal fungi: A tool for the conservation of endangered species. *Journal of Microbiological Methods*, 93, 134-7.

Flanagan, N.S., Otero J.T., Molineros F.H., Mosquera-Espinosa A.T, *et al.* (2012). Aprovechamiento sostenible de Recursos Biológicos Nativos del Distrito de Manejo Integrado de Atuncela. Guía Práctica. Cartilla de los Recursos Biológicos del Distrito de Manejo Integrado de Atuncela. Proyecto de CVC No 0051 y la Pontificia Universidad Javeriana-Cali. 31p.

Forero-Medina, G., & Joppa, L. (2010). Representation of global and national conservation priorities by Colombia's protected area network. *Plos One*, *5*(10), e13210.

Genaro JA. (2008). Origins, composition and distribution of the bees of Cuba (Hymenoptera: Apoidea: Anthophila). *Insecta Mundi* 52: 1-16.

Gentry, A.H. (1986). Species Richness and Floristic Composition of Choco region plant communities. *Caldasia* 15, 71-91.

Gillespie, T. W., Lipkin, B., Sullivan, L., Benowitz, D. R., Pau, S., & Keppel, G. (2012). The rarest and least protected forests in biodiversity hotspots. *Biodiversity and Conservation*, *21*(14), 3597-3611.

González, J.J., Etter, A.A., Sarmiento, A.H., Orrego, S.A., Ramírez, C., Cabrera, E., Vargas, D., Galindo, G., García, M.C. & Ordoñez, M.F. (2011). Análisis de tendencias y patrones espaciales de deforestación en Colombia. Instituto de Hidrología, Meteorología y Estudios Ambientales-IDEAM. Bogotá D.C., Colombia. 64 p.

González, M. & Paz, A. (2013). iBOL Colombia advances: Barcoding life in a megadiverse country. *Barcode bull*, 4 (2), 6.

González-Arnao M.T., Lázaro-Vallejo, C.E., Engelmann, F., Gómez-Pastrana, R., Martínez-Ocampo, Y.M., Pastelin-Solano, M.,C., *et al.* (2009). Multiplication and cryopreservation of vanilla (*Vanilla planifolia* 'Andrews'). *In Vitro Cellular & Developmental Biology - Plant - Springer*, 45, 574-82.

Hagsater, E., Dumont, V. & Pridgeon, A.M. (1996). Orchids. Status survey and conservation action plan. IUCN, Gland, Switzerland. 84 p.

Hernández-Hernández, J. (2011). *Vanilla* diseases. In: Handbook of *Vanilla* Science and Technology (pp. 26-39), First Edition. Edited by Daphna Havkin-Frenkel and Faith C. Belanger.

Householder, E., Janovec, J., Balarezo Mozambite, A., Huinga Maceda, J., Wells, J.& Valega, R. (2010). Diversity, natural history, and conservation of *Vanilla* (Orchidaceae) in Amazonian wetlands of Madre De Dios, Peru. *Journal of the Botanical Research Institute of Texas*, 4, 227 - 243.

Instituto de Hidrología, Meteorología y Estudios Ambientales de Colombia (IDEAM). (2015). Consulted 2-01-2016. http://www.ideam.gov.co/web/sala-de-prensa/noticias/-/asset_publisher/96oXgZAhHrhJ/content/aumenta-deforestacion-en-colombia-para-2014.

Jumpponen, A. & Trappe, J.M. (1998). Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytologist*, 140, 295-310

Koch, A.K., Fraga, C.N., Santos, J.U.M. & Ilkiu-Borges, A.L. (2013). Taxonomic Notes on *Vanilla* (Orchidaceae) in the Brazilian Amazon, and the Description of a New Species. *Systemetic Botany*, 38 (4), 975-981.

Khoyratty, S., Dupont, J. , Lacoste, S., Palama, T. ,Choi, Y ., Kim, H., *et al.* (2015). Fungal endophytes of *Vanilla planifolia* across Reunion Island: isolation, distribution and bio transformation. *BMC Plant Biology,* 15, 19. doi: 10.1186/s12870-015-0522-5

Koyyappurath, S., Atuahiva, T., Le Guen, R., Batina, H., Le Squin, S., Gautheron, N., Edel Hermann, V., Peribe, J., Jahiel, M., Steinberg, C., Liew, E. C. Y., Alabouvette, C., Besse, P., Dron, M., Sache, I., Laval, V. & Grisoni, M. (2015a). *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal agent of root and stem rot of vanilla. *Plant Pathology*, 65, 12445. doi: 10.1111/ppa.12445

Koyyappurath, S., Conéjéro, G., Dijoux, J.B., Lapeyre-Montès, F., Jade, K., Chiroleu, F., Gatineau, F., Verdeil, J.L., Besse, P. & Grisoni, M. (2015b). Differential Responses of *Vanilla* Accessions to Root Rot and Colonization by *Fusarium oxysporum* f. sp. *radicis-vanillae*. *Frontiers in Plant Science*, 6, 1125. doi: 10.3389/fpls.2015.01125

Liu, H., Ren, H., Liu, Q., Wen, X., Maunder, M. and Gao, J. (2015). Translocation of threatened plants as a conservation measure in China. *Conservation Biology*, 29, 1537–1551. doi: 10.1111/cobi.12585

Lubinsky, P., Cameron, K.M., Molina, M.C., Wong, M., Lepers Andrzejewski, S., Gómez-Pompa, A. & Kim S.C. (2008). Neotropical roots of a Polynesian spice: the hybrid origin of Tahitian vanilla, *Vanilla tahitensis* (Orchidaceae). *American Journal of Botany*, 95, 1040–1047.

Maxted, N., Ford-Lloyd, B. V. and Hawkes, J. G. (Eds). (1997). Plant conservation: The *in situ* approach. London: Chapman and Hall. 446 p.

Maxted, N., Dulloo, E., Ford-Lloyd, B.V., Iriondo, J.M., Jarvis, A. (2008) Gap analysis: a tool for complementary genetic conservation assessment. *Diversity and Distributions*, 14(6), 1018–1030.

Menchaca, R., Ramos, J.M., Moreno, D., Luna, M., Mata, M., Vázquez, L.M. & Lozano, MA. (2011). Germinación *in vitro* de híbridos de *Vanilla planifolia* y *V. pompona*. *Revista colombiana de biotecnología.* 13(1), 80-84.

Merritt, J.D., Hay, F.R., Swarts, N.D., Sommerville, K.D. & Dixon, K.W. (2014). *Ex Situ* Conservation and Cryopreservation of Orchid Germplasm. *International Journal of Plant Sciences*, 175(1), 46-58.

Ministerio de Ambiente y Desarrollo Sostenible y Universidad Nacional de Colombia (MADS & UNAL. (2015). Plan para el estudio y la conservación de las orquídeas en Colombia. Textos: Betancur, J., Sarmiento, H., Toro-González, L. & Valencia, J. Universidad Nacional de Colombia. Facultad de Ciencias. Instituto de Ciencias Naturales; Coord. Técnica: Higuera Díaz, Diego – Minambiente. Dirección de Bosques, Biodiversidad y Servicios Ecosistémicos Bogotá D.C.: Colombia. Ministerio de Ambiente y Desarrollo Sostenible; Universidad Nacional de Colombia. 336 p.

Molineros-Hurtado, F.H, González-Mina, R.T., Flanagan, N.S. & Otero, J.T. (2014). *Vanilla rivasii* (Orchidaceae), a new species from the Colombian pacific region. *Lankesteriana*, 13(3), 353-357.

Molineros-Hurtado, F.H. (2012). Caracterización morfológica y filogenia del género *Vanilla* en el distrito de Buenaventura-Valle del Cauca (Colombia). Tesis de Maestría en Ciencias Biológicas, Línea de Investigación Recursos Fitogenéticos Neotropicales. Universidad Nacional de Colombia, sede Palmira. 157 p.

Mosquera-Espinosa, A.T., Bayman, P. & Otero, J.T. (2010). *Ceratobasidium* como hongo micorrízico de orquídeas en Colombia. *Acta Agronómica*, 59, 316-326.

Mosquera-Espinosa, A.T., Otero, J.T., Molineros, F., Vásquez, E. & Flanagan, N.S. (2012). Bioprospección de los recursos nativos de la orquídea *Vanilla Spp*. presente en el Valle del Cauca. *Acta Agronómica*, 52(Especial), 37-38.

Mosquera-Espinosa A.T., Bayman, P., Prado,G., Gómez-Carabalí, A. & Otero, J.T. (2013). The double life of *Ceratobasidium*: orchid mycorrhizal fungi and their potential for biocontrol of *Rhizoctonia solani* sheath blight of rice. *Mycologia*, 105, 141-150.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (2000). Biodiversity Hotspots for Conservation Priorities. *Nature*, 403, 853-858.

Narum, S.R., Buerkle, C.A., Davey, J.W., Miller, M.R., Hohenlohe, P.A. (2013). Genotyping-by-sequencing in ecological and conservation genomics. *Molecular Ecology*, 22, 2841-7.

Ortiz-Valdivieso, P. (2015). *Vanilla*. In: R. Bernal, S.R. Gradstein & M. Celis (Ed.). Catálogo de plantas y líquenes de Colombia. Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá. http://catalogoplantascolombia.unal.edu.co

Otero, J.T., Mosquera-Espinosa, A.T. & Flanagan, N.S. (2013). Tropical orchid mycorrhizae: Potential applications in orchid conservation, commercialization, and beyond. *Lankesteriana* 13(1–2), 57-63.

Pansarin E.R. (2016). Recent advances on evolution of pollination systems and reproductive biology of Vanilloideae (Orchidaceae). *Lankesteriana* this volume

Pansarin, E. R., Aguiar, J. M. R. B. V. & Ferreira, A. W. C. (2012). A new species of *Vanilla* (Orchidaceae: Vanilloideae) from São Paulo, Brazil. Brittonia (Bronx, N.Y.), 64, 157–161.

Pansarin, E.R., Aguiar, J.M.R.B.V., Pansarin, L.M. (2014). Floral biology and histochemical analysis of *Vanilla edwallii* Hoehne (Orchidaceae: Vanilloideae): an orchid pollinated by *Epicharis* (Apidae: Centridini). *Plant Species Biology*, 29, 242-252. doi:10.1111/1442-1984.12014

Pansarin E.R. & Miranda M.R. (2016). A new species of *Vanilla* (Orchidaceae: Vanilloideae) from Brazil. *Phytotaxa* 267, 084-088.

Pinaria A.G., Liew, E.C.Y. & Burgess, L.W. (2010). *Fusarium* species associated with vanilla stem rot in Indonesia. *Australasian Plant Pathology*, 39, 176–183.

Pizano, C. & García H. (2014). El Bosque Seco Tropical en Colombia. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH). Bogotá, D.C. Colombia. 213 p.

Popova, E., Kim H.H., Saxena P.K., Engelmann, F. & Pritchard, H.W. (2016). Frozen beauty: The cryobiotechnology of orchid diversity. *Biotechnology Advances*. doi: 10.1016/j.biotechadv.2016.01.001

Porras-Alfaro, A. & Bayman, P. (2007). Mycorrhizal fungi of *Vanilla*: diversity, specificity and effects on seed germination and plant growth. *Mycology*, 99(4), 510 - 525.

Porras-Alfaro, A. & Bayman, P. (2011). Hidden Fungi, Emergent Properties: Endophytes and Microbiomes. *Annual Review Phytopathology*, 49, 291-315.

Ranadive, A.S. (2011). Quality control of *Vanilla* beans. In: Handbook of *Vanilla* Science and Technology (pp.141-161), First Edition. Edited by Daphna Havkin-Frenkel and Faith C. Belanger.

Reina-Rodríguez, G.A (ed.). (2011). *Vanilla odorata*. (pp. 45-55). En: Planes de manejo para la conservación de 22 especies focales de plantas en el departamento del Valle del Cauca, Cali, Colombia. 258p.

Roubik, D. W. & Ackerman, J. D. (1987). Long-term ecology of euglossine orchid bees (Apidae: Euglossini) in Panama. *Oecologia*, 73, 321-333.

Roux-Cuvelier, M. & Grisoni, M. I. (2010). Conservation and movement of *Vanilla* germplasm. In: Odoux, E. & Grisoni, M. (Ed.), *Vanilla* (pp. 31–41). Boca Ratón, FL: CRC Press.

Salazar-Rojas, V.M., Herrera-Cabrera, B.E., Delgado-Alvarado, A., Soto-Hernández, M., Castillo-González, F. & Cobos-Peralta, M. (2011). Chemotypical variation in *Vanilla planifolia* Jack. (Orchidaceae) from the Puebla- Veracruz Totonacapan region. *Genetic Resources and Crop Evolution*, 59, 875-887.

Schluter, P.M., Soto-Arenas, M.A. & Harris, S.A. (2007). Genetic variation in *Vanilla planifolia* (Orchidaceae). *Economic Botany*, 61, 328-336.

Seaton, P. & Pritchard, H.W. (2003). Orchid germplasm collection, storage and exchange (pp. 227-258). En: Dixon, K.W., S.P Kell, R.I Barrett & P.J. Cribb (eds.). Orchid conservation. Natural History Publications, Borneo.

Seaton, P.T. (2007). Establishing a global network of orchid seed banks. *Lankesteriana,* 7, 371-375.

Seaton, P.T., Hu, H., Perner, H. & Pritchard, H.W. (2010). *Ex situ* conservation of orchids in a warming world. *Botanic Review*, 76, 193-203.

Seaton, P. & Pritchard, H. (2011). Orchid seed stores for sustainable use. *Lankesteriana*, 11, 349-353.

Seaton, P., Kendon, J. P., Pritchard, H.W., Puspitaningtyas, D. M. & Marks, T.R. (2013). Orchid conservation: the next ten years. *Lankesteriana*, 13(1-2), 93-101.

Soto-Arenas, M.A. (2003). Vanilla. In: Pridgeon, A. M., Cribb, P. J., Chase, M. W. & Rasmussen F. N. [eds.]. (2003). Genera orchidacearum: Orchidoideae (pp. 321– 334).Oxford University Press, New York, New York, USA.

Soto-Arenas, M.A. (2006). La vainilla: retos y perspectivas de su cultivo. CONABIO. *Biodiversitas*, 66, 1-9.

Soto-Arenas, M.A. & Cribb, P. (2010). A new infrageneric classification and synopsis of the genus *Vanilla* Plum. Ex Mill. (Orchidaceae: Vanillinae). Lankesteriana, 9, 355-398.

Soto-Arenas, M.S., Dressler, R.L. (2010). A revision of the Mexican and Central American species of *Vanilla* Plumier ex Miller with a characterization of their ITS region of the nuclear ribosomal DNA. Lankesteriana, 9, 285-354.

Swarts, N. D. & Dixon, K. W. (2009a). Perspectives on orchid conservation in botanic gardens. *Trends in Plant Science*, 14, 590–598.

Swarts, N. D. & Dixon, K. W. (2009b). Terrestrial orchid conservation in the age of extinction. *Annals of Botany*, 104, 543–556.

Tan, B.C., Chin, C.F. & Alderson, P. (2010). Optimization of plantlet regeneration from leaf and nodal derived callus of *Vanilla planifolia* Andrews. *Plant Cell Tissue Organ Culture*, 105, 457-463.

Tremblay, R.L. & Hutchings, M.J. (2003). Population dynamics in orchid conservation: a review of analytical methods, based on the rare species *Lepanthes eltoroensis*. (pp. 183-204). En: Dixon, K.W., S.P Kell, R.I Barrett & P.J. Cribb (eds.). Orchid conservation. Natural History Publications, Borneo.

Van Dam, A. R., Householder, J. E. & Lubinsky, P. (2010). *Vanilla bicolor* Lindl. (Orchidaceae) from the Peruvian Amazon: autofertilization in *Vanilla* and notes on floral phenology. *Genetic Resources and Crop Evolution*, 57, 473-480.

van der Cingel, N.A. (2001). An atlas of orchid pollination: America, Africa, and Australia. A. A. Balkema, Rotterdam, Netherlands.

White, J. F., Torres, M.S., Sullivan, R.F., Jabbour, R. E., Chen, Q., Tadych, M., Irizarry, I., Bergen, M.S., Havkin-Frenkel, D. & Belanger, F.C. (2014). Occurrence of *Bacillus amyloliquefaciens* as a systemic endophyte of vanilla orchids. *Microscopy Research Technique*, 77, 874–885. doi: 10.1002/jemt.22410

Wood, C.B., Pritchard, H.W. & Miller, A.P. (2000). Simultaneous preservation of orchid seed and its fungal symbiont using encapsulation-dehydration is dependent on moisture content and storage temperature. *CryoLett* , 21, 125–36.

WWF Colombia (2008). Plan de Acción del Complejo

Ecorregional Chocó-Darién. Cali, Colombia. ISSB: 978-958-8353-04-3.

Zettler, L.W., Corey, L.L., Richardson, L.W., Ross, A.Y. & Moller-Jacobs, L. (2011). Protocorms of an epiphytic orchid (*Epidendrum amphistomum* A. Richard) recovered *in situ*, and subsequent identification of associated mycorrhizal fungi using molecular markers. *European Journal of Environmental Sciences*, 1, 108-114.

Zettler, L.W., Poulter, S.B., McDonald, K.I. & Stewart, S.L. (2007). Conservation-driven propagation of *Epidendrum nocturnum* (Orchidaceae) with a mycorrhizal fungi. *HortScience*, 42, 135–139.

LANKESTERIANA 16(2): 219–241. 2016.

doi: http://dx.doi.org/10.15517/lank.v16i2.26008

GENERA PLEUROTHALLIDINARUM: AN UPDATED PHYLOGENETIC OVERVIEW OF PLEUROTHALLIDINAE

ADAM P. KARREMANS

Lankester Botanical Garden, University of Costa Rica, P.O. Box 302-7050 Cartago, Costa Rica.
Naturalis Biodiversity Center, Leiden, The Netherlands • adam.karremans@ucr.ac.cr

ABSTRACT. Subtribe Pleurothallidinae with just over 5000 species is possibly the most species-rich of all orchids. It has been growing steadily for more than two centuries, but the last three decades have been especially active in terms of systematic and phylogenetic studies in the group. The growth in species numbers has been accompanied by the marked increase in generic and infrageneric concepts. Nevertheless, Pleurothallidinae are plagued with cases of convergent and divergent morphology, and phylogenetic relatedness is not always apparent. This opens the door to controversial changes in generic circumscriptions that are considered too inclusive by some and too exclusive by others. A grave consequence of these disagreements is the difficulty of assessing which and how many species actually belong to each genus. Here an attempt is made to place generic names among their close relatives as a first step to re-evaluating the whole subtribe.

KEY WORDS: *Acianthera*, *Anathallis*, *Dilomilis*, *Masdevallia*, *Octomeria*, *Phloeophila*, *Pleurothallis*, *Restrepia*, *Specklinia*, systematics

Introduction. *Pleurothallis* R.Br., the type genus of subtribe Pleurothallidinae Lindl., was described more than two centuries ago. Historically, *Pleurothallis* and Pleurothallidinae have been treated almost as synonyms, with the exclusion of only a few morphologically well-recognizable genera from *Pleurothallis* over the centuries. The first systematic classification of the members of Pleurothallidinae is possibly that of Lindley (1842, 1859). Several authors followed with additional proposals to tackle *Pleurothallis* and its segregate genera (i.e. Reichenbach, Barbosa Rodrigues, Cogniaux, Schlechter, Garay, Dressler, and others). Members of the genus had, however, not undergone as many changes as they have in the last three decades. Luer's first monographs of the group in 1986 (Luer 1986a, 1986b, 1986c) triggered a proliferation of systematic studies in the subtribe which would have been impossible before. Dozens of monographs followed. The first molecular phylogeny was published by Pridgeon, Solano and Chase in 2001 and was followed by a proposal to redefine the whole subtribe (Pridgeon & Chase 2001). However, the significant systematic and taxonomic changes proposed after that, in addition to the rapid increase in species numbers within Pleurothallidinae, has more than ever fueled the need for a comprehensive picture of phylogenetic relationships within the subtribe.

This issue with the classification of the megadiverse *Pleurothallis* (in a traditional sense) has historically been the same one: the realization that it is not monophyletic but that there was no consistent way to resolve the systematics of the group with the available data. In 1859, Lindley said about *Pleurothallis* "I think it necessary to preserve this great and difficult genus without dismemberment. Not that I regard it as a really single aggregation of species...." A century later Luer himself would state that "*Pleurothallis* is indeed capable of being divided, but because of the various interrelationships, most divisions at the subgeneric and sectional levels seem more practical" and added, "A *Pleurothallis* might be described as any pleurothallid that does not fit into any of the other genera" (Luer 1986c). After their morphologically based cladistic study of the group, Neyland *et al.* (1995) also noted that "the large genus *Pleurothallis* is polyphyletic and, therefore, may be divided into several genera", while in his palynological study of the subtribe Stenzel (2000) would suggest that "the genus comprises a combination of morphologically rather underived sister taxa of the other pleurothallid genera". But, it was not until Pridgeon and Chase

Received 4 May 2016; accepted for publication 21 August 2016.

(2001), based on the molecular studies by Pridgeon *et al.* (2001), that *Pleurothallis* was finally dismembered. The authors noted that it "has been nothing but a polymorphic assemblage for almost two centuries" and that "many taxa with conspicuous autapomorphies were segregated from it, gradually leaving the genus itself with no defining synapomorphies."

Pridgeon and Chase (2001) argued that "many characters are difficult to score in cladistic analyses because they are either continuous or probably not homologous. These same characters show up repeatedly in his [Luer's] artificial key to the subgenera." Nevertheless, the complexity of the group and their limited sampling size forced them to admit that "for nomenclatural transfers we extrapolated from the study taxa to morphologically similar taxa as recognized by Luer." Not surprisingly, subsequent phylogenetic studies within the Pleurothallidinae have shown that the generic, subgeneric and sectional systematics of the subtribe were not fully resolved. Re-circumscriptions and emendations were either made or at least suggested by several authors who used novel analytical methods and/or included a broader sampling of species (Stenzel 2004, Abele 2007, Karremans 2010, 2014, Chiron *et al.* 2012, Karremans *et al.* 2013a, 2013b, Wilson *et al.* 2013, Karremans & Rincón-González 2015, Chiron *et al.* 2016, Karremans *et al.* in press). Meanwhile, hundreds of species' names, either new species or combinations, and dozens of new genera have since then been proposed by Luer (2002a, 2004, 2005, 2006, 2007, 2009) and others, mostly but not exclusively, on the basis of morphology.

There is a pressing need for reviewing the phylogenetic relationships of many groups within the pleurothallids. Nowadays authors are frequently compelled by editors and reviewers to use so-called "widely accepted" names. Those names frequently follow particular databases or comprehensive rather than specialized monographic works. Although this tendency might be understandable, it is not in the best interest of the scientific community. If one were to follow the WCSP, to cite an example of a database used as a reference by several journals, one would soon get into trouble. Under the synonymy of genus *Pleurothallis*, for example, are many genera that we know are not even closely related. The list includes *Antilla* (Luer) Luer, *Apoda-prorepentia* (Luer) Luer and *Pleurobotryum* Barb.Rodr., phylogenetically related to *Acianthera*; it also lists *Areldia* Luer, *Cucumeria* Luer, *Gerardoa* Luer and *Rubellia* (Luer) Luer, which are actually *Specklinia* Lindl. relatives; *Andreettaea* Luer, with unknown affinity, and *Sansonia* Chiron, basal in the pleurothallids, are also placed under *Pleurothallis* even though clearly not related to that genus. Most of these genera have been argued and proven to be distinct in different papers published mostly after *Genera Orchidacearum* (Pridgeon 2005). The basis for their establishment has been morphological or molecular, and sometimes both. The issue with Pleurothallidinae goes much further than choosing a particular classification system. Different classification systems should be alternative, but never conflicting. Say that system A proposes a single genus (1), whereas system B proposes two genera (1 and 2). In alternative proposals the same species are included in each system; in system A all of them are included in a single genus (genus 1), and in system B the same species are segregated into two genera (1 and 2). This means that the formula A1 = B1 + B2 is true. In contrast, in conflicting proposals that formula does not hold true. For example, the monophyletic genus *Phloeophila* (sensu Pridgeon 2005) could be otherwise interpreted as the sum of the also monophyletic genera *Luerella* Braas, *Ophidion* Luer and *Phloeophila* Hoehne & Schltr. These proposals are therefore alternative. However, the generic concept of *Specklinia* (sensu Pridgeon 2005) is not equal to the sum of *Acostaea* Schltr., *Empusella* (Luer) Luer, *Gerardoa*, *Muscarella* Luer, *Sarcinula* Luer, *Specklinia* and *Sylphia* Luer (sensu Luer 2006), because many species included by one were excluded by the other. Unfortunately most current generic circumscriptions in the pleurothallids are conflicting. This is also the reason why it is difficult to determine how many accepted species the subtribe has, let alone how many accepted species belong in each genus.

It is to be expected that more inclusive generic concepts are more likely to be monophyletic than narrow generic concepts. That, together with fact that broader generic concepts will intrinsically reduce the number of generic names to be used, has led the community to prefer a conservative, more inclusive, approach. This is especially true when so called "splinter" genera are neither phylogenetically placed nor proven monophyletic. Nevertheless, broad is not always better. In a world where species can only be placed among their close relatives using DNA barcoding, students and hobbyists alike are faced with the everyday problem of not being able to place the right name tags on their plants. When genera are extremely inclusive and lack distinct morphological synapomorphies they become undiagnosable. It is made worse when no subgeneric classification is proposed. In that sense, information is key. Every species should be placed in a discrete grouping, be it at generic or subgeneric level, making it possible to find that species, and its relatives. In turn, each grouping should convey underlying information about the evolutionary history, distribution, ecology, etc. about the species assigned to it. They are otherwise useless.

In the midst of preparing a comprehensive systematic treatment of the Pleurothallidinae (Karremans, in prep.), an overview of the major affinities within the subtribe is presented here.

Subtribe **Pleurothallidinae** Lindl.
(Edward's Bot. Reg. 15: tab. 1298, 1829, *nom. nud*); Gen. Sp. Orch. Pl. 3. 1830.

Type: *Epidendrum ruscifolium* Jacq., Enum. Pl. Carib. 29, 1760. [= *Pleurothallis ruscifolia* (Jacq.) R.Br. in Aiton, Hort. Kew. ed. 2, 5: 211. 1813.]

Ety.: From the Greek *pleurothallos*, "riblike branches" referring to the caespitose, slender ramicauls found in *P. ruscifolia* and in most of the Pleurothallidinae.

Plants perennial, epiphytic to lithophytic. Rhizome abbreviated to repent. Roots velamentous. Ramicauls non-pseudobulbous, cylindric, sheathed, mostly unifoliate. Leaf coriaceous, conduplicate, articulate. Inflorescence lateral or terminal, rarely basal on the stem, racemose, frequently successive. Flowers generally resupinate. Ovary articulated with the pedicel, cylindric. Column stout or elongate, subterete, the base footless to having a prominent foot. Anther dorsal, apical or ventral. Stigma apical or ventral, solitary, lobed. Pollinia 2, 4, 6 or 8, suborbicular to ovoid, rarely without caudicles, sometimes also with a viscidium. Fruits ellipsoid.

Generalities. In 1986, Luer accounted for as many as 4000 species in subtribe Pleurothallidinae. About two decades later Pridgeon (2005) increased the number to 4100 species. Today, 30 years after Luer's monograph, just over 5100 species (5114 today to be precise) are currently being accepted among the 12,000 published names applicable to the subtribe (Karremans, in prep.; Table 1). This number will probably increase by a few hundred in the next couple of decades as species complexes are studied more carefully and more literature on the Pleurothallidinae becomes available. As treated here, *Lepanthes* and *Stelis* each represent just over 20% of the species belonging to the subtribe, both with just above 1000 species (Fig. 1). *Masdevallia* and *Pleurothallis* each represent above 10% of the subtribe, both with around 500-600 species. There are eight other genera with more than 100 species each: *Acianthera*, *Anathallis*, *Dracula*, *Octomeria*, *Pabstiella*, *Platystele*, *Specklinia* and *Trichosalpinx*; they represent from 2% to 6% of the subtribe.

A major challenge within Pleurothallidinae is finding and defining phylogenetically informative, synapomorphic, morphological features. The recent molecular-based phylogenies have shown that many traditionally considered diagnostic morphological characters have appeared more than once independently

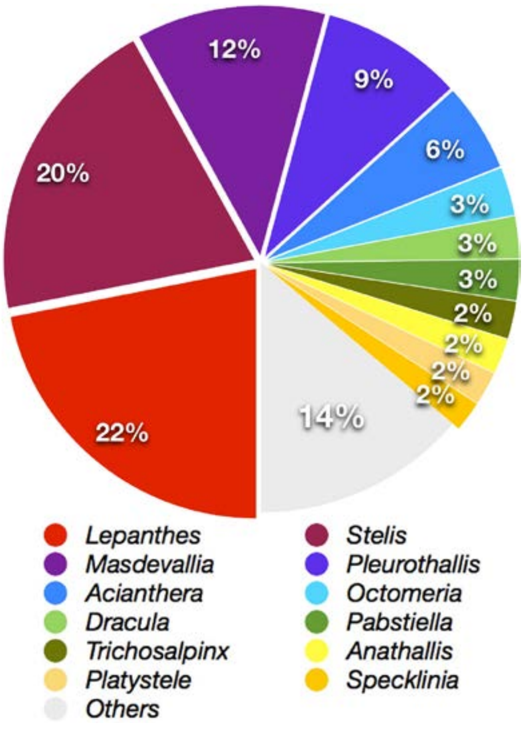


Figure 1. Relative species richness per genus in the Pleurothallidinae as currently defined.

TABLE 1. Accepted number of species per genus in the Pleurothallidinae at the time of preparation of this manuscript (Karremans, in prep.).

Genus	Species Number	Genus	Species Number
Acianthera	291	*Muscarella*	54
Anathallis	116	*Myoxanthus*	49
Andinia	71	*Neocogniauxia*	2
Andreettaea	1	*Octomeria*	159
Atopoglossum	3	*Pabstiella*	138
Barbosella	21	*Phloeophila*	9
Brachionidium	79	*Platystele*	110
Chamelophyton	1	*Pleurothallis*	465**
Dilomilis	5	*Pleurothallopsis*	18
Diodonopsis	5	*Porroglossum*	52
Dondodia	1	*Restrepia*	55
Draconanthes	2	*Restrepiella*	3
Dracula	134	*Sansonia*	2
Dresslerella	13	*Scaphosepalum*	50
Dryadella	57	*Specklinia*	100
Echinosepala	12	*Stelis*	1027***
Frondaria	1	*Teagueia*	14
Kraenzlinella	11	*Tomzanonia*	1
Lankesteriana	21	*Trichosalpinx*	123
Lepanthes	1120	*Trisetella*	24
Lepanthopsis	44	*Zootrophion*	26
Madisonia	1		
Masdevallia	623*	**TOTAL**	**5,114**

*At the time of preparation of this manuscript the total species number for genus *Masdevallia* had not been revised thoroughly.
**The number of species attributed to *Pleurothallis* excludes those names that have not been placed elsewhere but most like do not belong in the genus. At least a few dozen names can be attributed to a so called "unplaced" category at this time.
***More than 80 additional species of *Stelis* are being described in the next volume of Luer's Icones Pleurothallidinarum, *Stelis* of Bolivia, which is in press. Those are not included in this species count at this time.

in different lineages of the subtribe. It is to be expected that in such a large and diverse group, single characters are not completely unique; nevertheless that does not mean they are uninformative, especially when used in combination. This is especially the case for groups of species that under specific pollinator pressure have evolved having similar floral morphology even though they are not phylogenetically related. Luer (1986a) noted that plant morphology, which is not under such pressure, can be a better indicator of common evolutionary history. Nonetheless, plant morphology can also be under selective pressure due to particular ecological conditions, and cannot therefore be used by itself either. Aside from understanding if characters are phylogenetically informative or not, whether a particular state is ancestral or derived has traditionally been one of the most difficult to determine. Luer (1986a) could not with certainty determine whether eight pollinia or two pollinia is the most ancestral state in Pleurothallinae. DNA-based phylogenies have helped in establishing not only how many times certain morphological features have appeared but also the polarity of their states.

There are nine major clades within the Pleurothallidinae (Fig. 2). For ease of discussion, all generic and subgeneric groupings within the subtribe are grouped by their affinity to one of the genera within each of those clades. The proposed affinities are *Acianthera* (Ac), *Dilomilis* (Di), *Lepanthes* (Le), *Masdevallia* (Ma), *Octomeria* (Oc), *Phloeophila* (Ph), *Pleurothallis* (Pl), *Restrepia* (Re) and *Specklinia* (Sp); the oldest generic name is used here for the species within those clades (except *Pleurothallis*). This phylogenetic arrangement of the subtribe, despite taking only DNA-based studies into consideration (Pridgeon *et al.* 2001, Stenzel 2004, Solano-Gómez 2005, Matuszkiewicz & Tukallo 2006, Abele 2007, Forster 2007, Meyer & Cameron 2009, Karremans 2010, 2014, Endara 2011, Bogarín *et al.* 2013, Chiron *et al.* 2012, Karremans *et al.* 2013a, 2013b, Wilson *et al.* 2013, in press, Pessoa *et al.* 2014, Karremans & Rincón-González 2015, McDaniel & Cameron 2015, Chiron *et al.* 2016), has

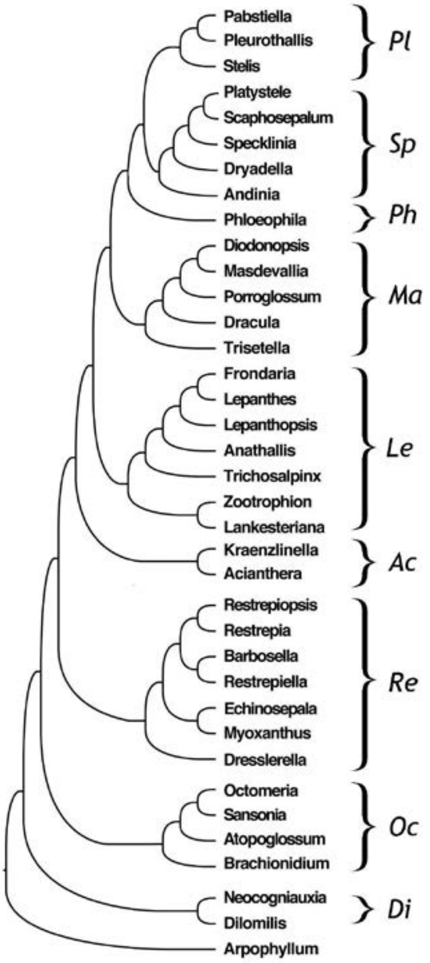


Figure 2. Phylogenetic overview of the main affinities within the Pleurothallidinae based on the available DNA based studies.

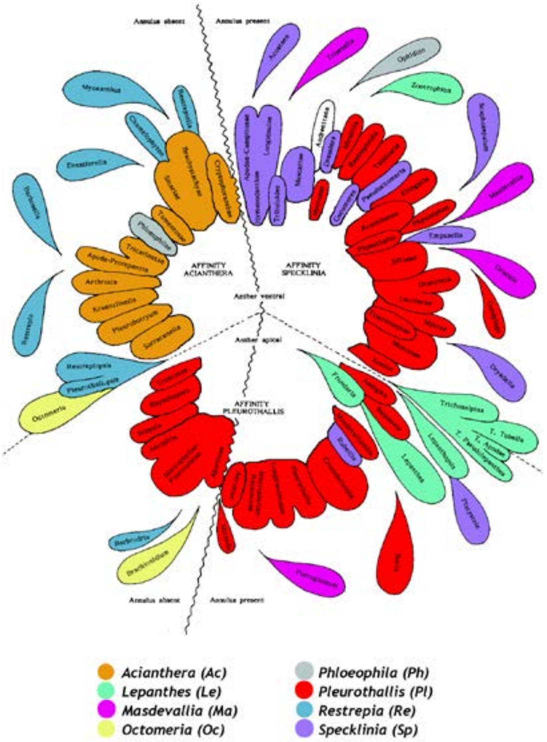


Figure 3. The affinities within the Pleurothallidinae proposed here superimposed on the morphologically based phylogenetic inference of the subtribe proposed by Luer (1986c).

striking similarities with that proposed by Luer (1986c) on the basis of morphology (Fig. 3). Nevertheless, it is important to note at this point that convergence in basically all morphological traits that have been given phylogenetic importance are known to occur in the subtribe. The phylogenetic inference proposed by Neyland *et al.* (1995) based mostly on anatomical characters of the leaf, stem, and root is extremely different from that presented by Stenzel (2000) based on pollen morphology; both differ substantially from Luer (1986c), which was based mostly on anther position, lateral sepal fusion, and presence or absence of an annulus. None of them reflects the DNA-based phylogenetic inferences faithfully.

Affinity *Acianthera*

Several generic names have been proposed within this group, most quite recently. However, only *Acianthera* (Fig. 4) and *Kraenzlinella* currently receive broad acceptance as distinct genera. Both genera, although proposed more than a century ago, were not commonly used until recently when they were re-established on the basis of the DNA analyses published by Pridgeon *et al.* (2001). DNA data made available of additional species of both genera, as well as of other species of *Pleurothallis* (from which they were segregated), show that they are still in need of redefinition. If *Acianthera* is to be maintained in its currently most accepted, broad sense (Pridgeon 2005), then it includes the generic concepts of *Arthrosia*, *Brenesia*, *Cryptophoranthus*, and *Sarracenella*, as proposed by Pridgeon & Chase (2001) and Chiron & van den Berg (2012). Nevertheless, it should also include *Aberrantia*, as suggested by Luer (2004) and

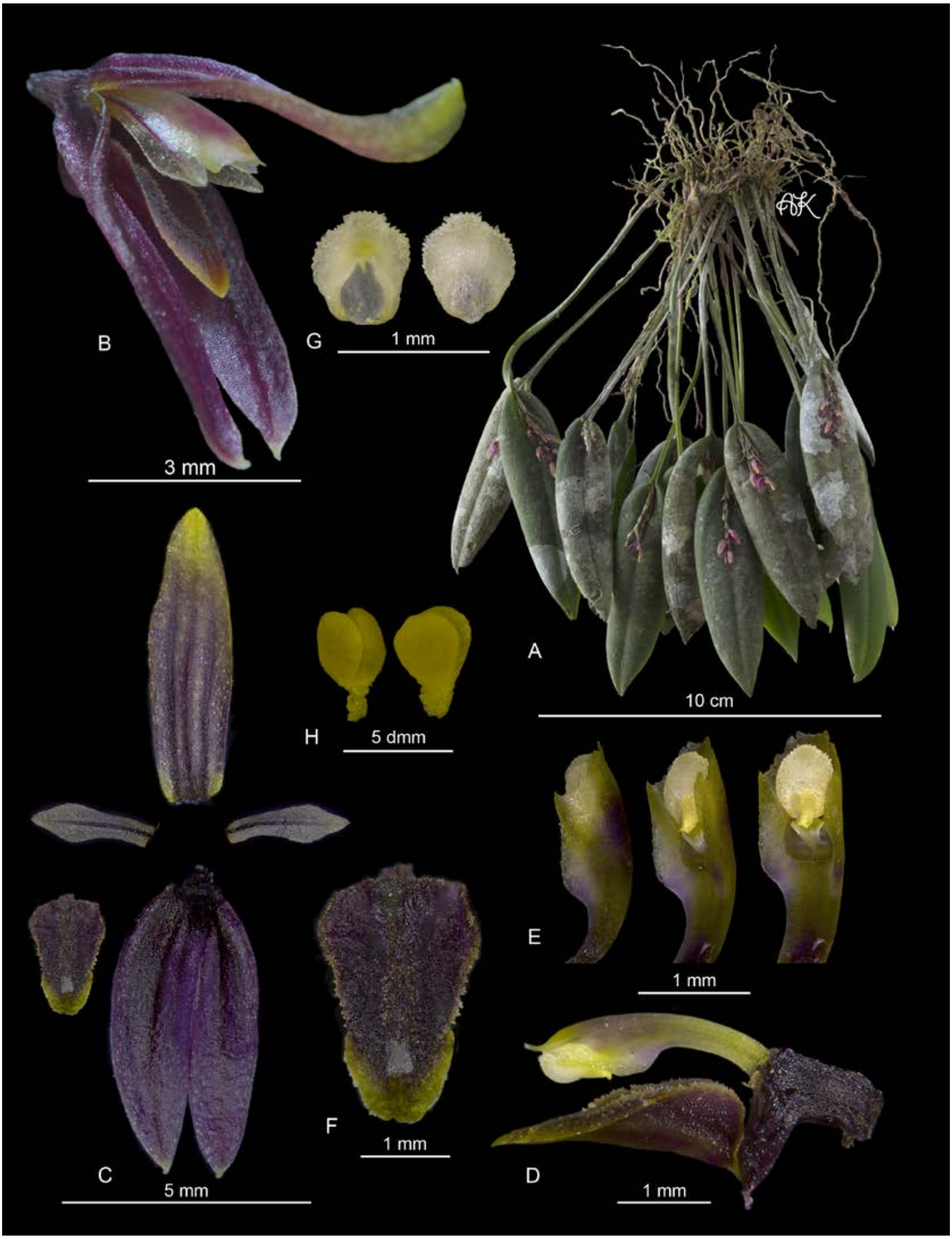


FIGURE 4. Lankester Composite Dissection Plate (LCDP) of *Acianthera lojae* (Schltr.) Luer. A. Habit. B. Flower. C. Dissected perianth. D. Column and lip, lateral view. E. Column in ventral and lateral views. F. Lip G. Anther cap. H. Pollinia. Photographs by Andrea Morales Morales and A. Karremans based on *Karremans 6548* (JBL-spirit).

proposed by Bogarín *et al.* (2008), *Antilla*, as shown by Stenzel (2004), *Apoda-prorepentia*, as shown by Stenzel (2004) and Karremans & Rincón-González (2015), *Didactylus*, as suggested by Luer (2004), *Ogygia*, as proposed by Solano-Gómez (2003; 2015), *Pleurobotryum*, as shown by Chiron *et al.* (2012), *Proctoria*, as suggested by Luer (2006), and *Unguella*, as suggested by Luer (2004). To my knowledge, there are no DNA data available (published or unpublished) of species belonging to the monospecific genera *Ogygia* and *Proctoria* that can confirm their exact phylogenetic relationships. However, the first is similar to other *Acianthera* species found in Mexico and Guatemala and the second is reminiscent of *Antilla* species, as was also noted by Stenzel (2007), and as such it would belong in *Acianthera* s.l.

Kraenzlinella, as previously mentioned, has lately received recognition as a distinct genus (Pridgeon 2005). In the molecular phylogeny of Pleurothallidinae published in Pridgeon *et al.* (2001), the single *Kraenzlinella* sequence was found sister to *Brachionidium*, and not particularly closely related to the other *Acianthera* species. Nevertheless, the availability of additional sequences from diverse *Acianthera* species (Stenzel 2004, Chiron *et al.* 2012, Karremans & Rincón-González 2015) and also from diverse *Kraenzlinella* species now places the genus close to *Acianthera*. This is expected, and was indeed mentioned by Luer (1994), based on morphological similarity; *Brachionidium* is quite different. In fact, it is likely that the relatively long branches of the representatives of both *Kraenzlinella* and *Brachionidium* had initially misplaced the two genera in Pridgeon *et al.* (2001), as neither is related to *Myoxanthus* as was found. *Kraenzlinella* is for now accepted as it was defined by Pridgeon (2005) until more evidence is available, but it is likely that it will need to be either redefined or included within a broader concept of *Acianthera*.

Finally, genus *Dondodia* Luer was proposed on the basis of *Cryptophoranthus erosus* Garay, an unusual species known only from Hispaniola. It had been placed in *Pleurothallis* subgen. *Acianthera* sect. *Cryptophoranthae* Luer (1986c) on account of the connation of the dorsal sepal with the lateral synsepal. The species of that section would later be found embedded within *Acianthera* (Pridgeon *et al.* 2001) and transferred to the genus (Pridgeon and Chase 2001), where they were later given subgeneric recognition as *Acianthera* sect. *Cryptophoranthae* (Luer) Chiron & van den Berg. Even though it is likely that *Dondodia erosa* (Garay) Luer does belong in the *Acianthera* affinity, it is unclear where it is placed. It is perhaps related to species in the Antillean genera *Antilla* and *Proctoria*.

Affinity *Dilomilis*

This affinity is made up of only three genera, *Dilomilis* Raf., *Neocogniauxia* Schltr. and *Tomzanonia* Nir, of which eight species are known. They have been mostly associated with Laeliinae until they were found sister to Pleurothallidinae in the DNA-based phylogeny presented by Pridgeon *et al.* (2001). Thereafter, *Dilomilis*, *Neocogniauxia,* and *Tomzanonia* have been treated as members of Pleurothallidinae by Pridgeon (2005). It is clear, nevertheless, that they are at best aberrant among the pleurothallids, and the fact is that the same DNA data would also justify their placement as a sister subtribe alongside rather than within Pleurothallidinae.

It is important to note that when describing genus *Dilomilis*, Rafinesque described and selected as type a species with the name *D. serrata* Raf. Diverse authors have ascribed that name solely to Rafinesque as if the author were proposing it as a new species. Nevertheless, Rafinesque's intention was clearly to transfer *Octomeria serratifolia* Hook. to *Dilomilis* as he specifically cited Hooker's original publication and literally transcribed the original Latin description and collection data. The name was simply misspelled, a misspelling that is to be corrected and does not render his combination in *Dilomilis* invalid. Therefore, that name should correctly be cited as *Dilomilis serratifolia* (Hook.) Raf., and as a consequence the genus *Octadesmia* is illegitimate because it based on the same type species as *Dilomilis*.

The exclusion of the genera in the *Dilomilis* affinity from Pleurothallidinae, as Borba *et al.* (2011) pointed out, allows for a better characterization of the latter. Traditional diagnostic morphological synapomorphies for Pleurothallidinae such as 1) presence of a stem transformed into a monophyllous ramicaul, and 2) an articulation between the pedicel and ovary, together with biological synapomorphies, including 3) autoincompatibility and 4) myophily (fly pollination), are lost with the inclusion of species of *Dilomilis*, *Neocogniauxia,* and *Tomzanonia*. Nevertheless, DNA data clearly show that they are closer to Pleurothallidinae than to any other subtribe.

Affinity *Lepanthes*

Eight genera make up this affinity: *Anathallis* Barb.Rodr., *Draconanthes* (Luer) Luer, *Frondaria* Schltr., *Lankesteriana* Karremans, *Lepanthes* Sw., *Lepanthopsis* (Cogn.) Ames, *Trichosalpinx* Luer, and *Zootrophion* Luer. Of these, only *Lepanthes* and *Lepanthopsis* received generalized recognition in botanical literature before Luer's monographic works on Pleurothallidinae. In addition to accepting the former, Luer proposed *Frondaria*, *Trichosalpinx* and *Zootrophion* in the 1980s, and *Draconanthes* in the 1990s. They received recognition by other authors as well and were retained as such by Pridgeon and Chase (2001) and Pridgeon (2005). *Anathallis*, which had traditionally been considered a synonym of *Pleurothallis*, would be re-considered on the basis of DNA evidence presented by Pridgeon et al. (2001). *Anathallis* as defined then was latter shown to include a group of species more closely related to some *Trichosalpinx* (Chiron *et al.* 2012; Karremans 2014), for which genus *Lankesteriana* was proposed, and a group of species belonging to *Stelis* s.l. (Chiron et al. 2012; Karremans et al. 2013a). The exclusion of those taxa from *Anathallis* rendered it monophyletic (Karremans 2014).

After the DNA-based redefinition of Pleurothallidinae, three more genera belonging to the *Lepanthes* affinity would be proposed on morphological grounds: *Epibator* Luer, *Expedicula* Luer, and *Panmorphia* Luer. In general terms they received little acceptance. *Epibator* was segregated from *Zootrophion*, and in fact DNA data showed that *Epibator* species form a monophyletic group sister to the other species of *Zootrophion*. Nevertheless, genetic distance is low. *Expedicula* was proposed to segregate two species previously placed in *Lepanthopsis*. Unpublished DNA data found *Expedicula* species embedded within the latter, it cannot therefore be recognized without rendering *Lepanthopsis* polyphyletic. *Panmorphia*, as the name suggests, was conceived as polyphyletic. Luer (2009) himself would note that many of its species graded into *Anathallis* and would place it in synonymy. DNA data showed that indeed the type species of *Panmorphia*, *Anathallis sertularioides* (Sw.) Pridgeon & M.W.Chase, is a close relative of the type of *Anathallis*, *Anathallis obovata* (Lindl.) Pridgeon & M.W.Chase (Stenzel 2004, Chiron *et al.* 2012, Karremans 2014).

Trichosalpinx is a special case in Pleurothallidinae because it is clearly non-monophyletic, but there seems to be no way to resolve the issue at this time. Luer's 1997 monograph of *Trichosalpinx* already suggested that the genus was artificial as it was made up basically of any pleurothallid species with lepanthiform bracts that did not fit the criteria of *Draconanthes*, *Lepanthes* or *Lepanthopsis*. In both Pridgeon *et al.* (2001) and Karremans (2014), *Trichosalpinx* was found polyphyletic, but no proposals to re-circumscribe it were made. Unpublished DNA data from three out of the four subgenera proposed by Luer (1997) indicate that *Trichosalpinx* contains at the very least seven unrelated clades, and that they are diversely interrelated to all of the other accepted genera in this affinity. Therefore, *Trichosalpinx* will certainly need to be dismembered and its species either assigned to broader concepts of currently accepted genera and/or to novel generic concepts.

Affinity *Masdevallia*

Five genera belonging to this affinity have been recognized with some consistency in the last few years: *Diodonopsis* Pridgeon & M.W.Chase, *Dracula* Luer, *Masdevallia* Ruiz & Pav. (Fig. 5), *Porroglossum* Schltr. and *Trisetella* Luer. *Masdevallia* was proposed in the 18th century, and it was not until the beginning of the 20th century that the first group of species would be segregated from it under the generic name *Porroglossum*. In the second half of the 20th century, Luer proposed the segregation of three more species groups from *Masdevallia* under the generic names *Dracula*, *Dryadella* Luer (which does not belong to this affinity), and *Trisetella*. The five genera would be recognized and kept separate from *Masdevallia* in the following works (Luer 1986a, 1986b), and all of them would receive support as distinct, monophyletic genera in the molecular phylogeny published by Pridgeon *et al.* (2001). From this point on the story becomes much more complex.

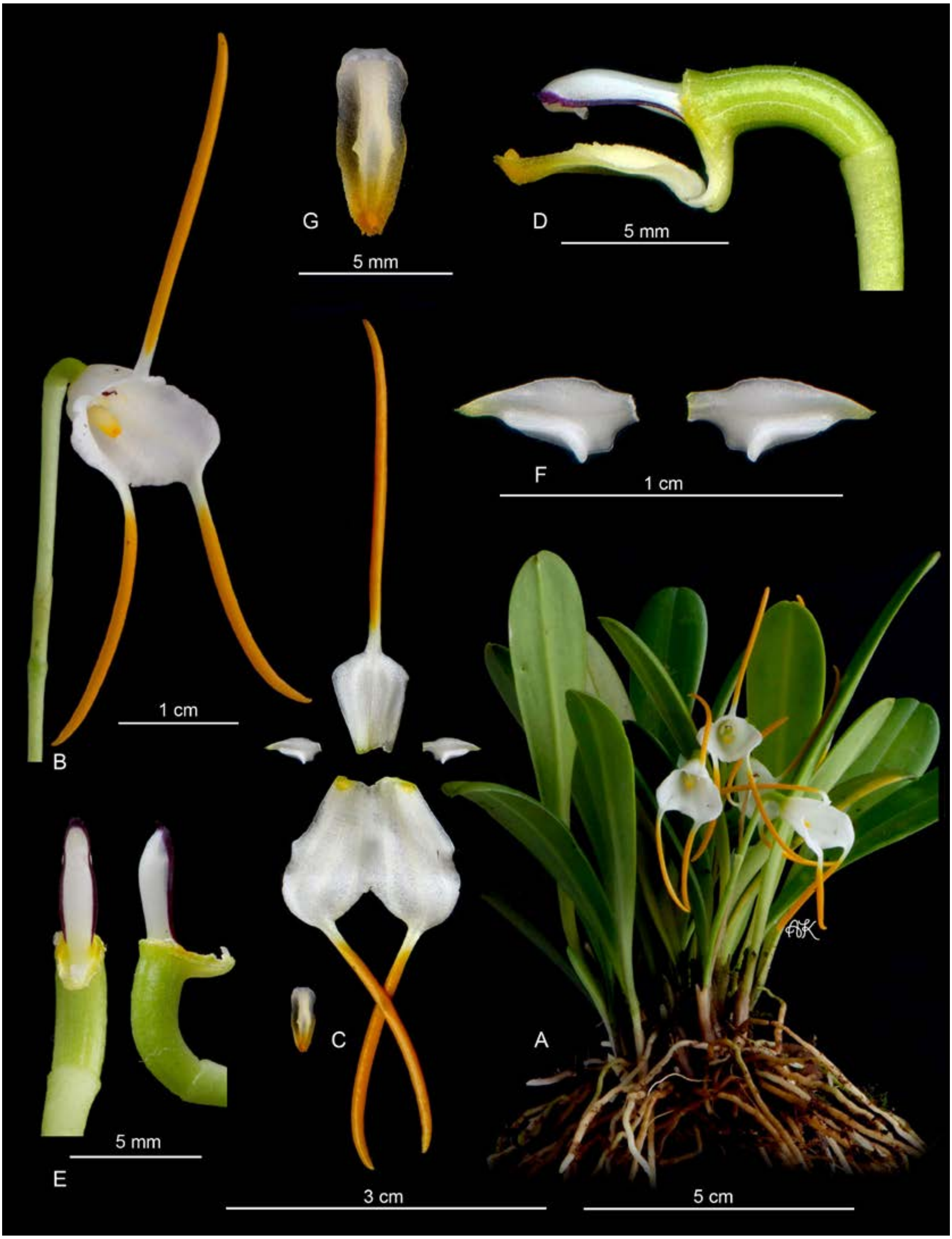


Figure 5. Lankester Composite Dissection Plate (LCDP) of *Masdevallia laucheana* J.Fraser. A. Habit. B. Flower. C. Dissected perianth. D. Column and lip, lateral view. E. Column in ventral and lateral view. F. Petals. G. Lip. Photographs by A. Karremans based on *Karremans 5901* (JBL-spirit).

Pridgeon and Chase (2001) proposed genus *Diodonopsis* for what was previously *Masdevallia* sect. *Pygmaeae* (Luer 1986b), based on the solitary placement of the accession of the single species of this group included in their molecular phylogeny (Pridgeon *et al.* 2001). Prior to the proposal, Luer (2000b) had suggested that the species belonging to his *Masdevallia* subgen. *Pygmaeia* "probably are not closely related," and doubted the monophyly of *Diodonopsis*, pointing out that the type species had not been analyzed (Luer 2002b). In fact, later DNA studies that included more samples of *Diodonopsis* and *Masdevallia* (Matuszkiewicz & Tukallo 2006, Abele 2007) proved that indeed the former is not monophyletic as currently defined. Nevertheless, it does appear that *D. erinacea* (Rchb.f.) Pridgeon & M.W.Chase, the single species originally analyzed, and *D. pygmaea* (Kraenzl.) Pridgeon & M.W.Chase, the type species of the genus, are sister to each other. What is not yet clear is how many other species actually belong in *Diodonopsis* and if the genus is truly distinct from *Masdevallia*.

Luer would not leave the matter at that and a few years later proposed additional segregate genera from *Masdevallia: Acinopetala* Luer, *Alaticaulia* Luer, *Buccella* Luer, *Byrsella* Luer, *Fissia*, *Jostia* Luer, *Luzama* Luer, *Megema* Luer, *Petalodon* Luer, *Regalia* Luer, *Reichantha* Luer, *Spectaculum* Luer, *Spilotantha* Luer, *Streptoura* Luer, *Triotosiphon* Schltr. ex Luer, and *Zahleria* Luer, as well as the recognition of the previously proposed *Jostia* Luer, *Portillia* Königer and *Rodrigoa* Braas (Luer 2006). The proposals received little acceptance from other authors because of the lack of accompanying evidence. In fact, the available DNA data show that most of these ill-defined genera are indeed artificial as currently circumscribed (Pridgeon *et al.* 2001, Matuszkiewicz & Tukallo 2006, Abele 2007).

Affinity *Octomeria*

Affinity *Octomeria* as defined here is made up of four genera, *Atopoglossum* Luer, *Brachionidium* Lindl., *Octomeria* R.Br. (Fig. 6) and *Sansonia* Chiron. *Octomeria*, proposed early in the 19th century to accommodate pleurothallid species with eight pollinia, has remained virtually unchanged since it was published. The same can be said for *Brachionidium*, a genus of species with 6 or 8 pollinia and mostly recognized as a distinct genus since it was proposed in 1859. *Atopoglossum*, published in 2004, was established for a few Cuban endemic species with an abbreviated ramicaul, a three-lobed lip, an elongate column, and eight pollinia. *Sansonia*, published in 2012, was named on the basis of the lone placement of *Pleurothallis neobradei* Luer and an unnamed *Acianthera* species in a DNA-based phylogenetic analyses of Brazilian pleurothallids.

The phylogenetic study of Pleurothallidinae based on DNA data (Pridgeon *et al.* 2001) showed *Octomeria* in a basal position in the subtribe. That in fact made sense considering that the morphological features of *Octomeria*, elongate stems and multiple pollinia, are somewhat reminiscent of *Dilomilis* and *Neocogniauxia*, the sister genera of the Pleurothallidinae. *Brachionidium* however, was found sister to *Kraenzlinella*, in a clade that was sister to *Myoxanthus*. Morphologically it makes little sense that *Brachionidium*, *Kraenzlinella* and *Myoxanthus* are close relatives; in fact here they are treated in three different affinities. DNA-based phylogenetic analyses by Stenzel (2004) and Forster (2007) place *Brachionidium* in a clade together with *Atopoglossum* and *Octomeria*. My own analyses also place *Brachionidium* sister to those same genera. It is worth mentioning that the sequences of *Brachionidium* species include many unique changes (indels) which make them difficult to align and which results in them having extraordinarily long branches. That makes them difficult to place with complete certainty. Nevertheless, the placement of *Brachionidium* close to *Atopoglossum* and *Octomeria*, rather than *Kraenzlinella* and *Myoxanthus*, is much more likely based on morphology, but this remains to be confirmed. The species of all three genera have 6 or 8 pollinia.

The two species of *Sansonia* were found to be sister to all Pleurothallidinae when using *Octomeria* as outgroup (Chiron *et al.* 2012). However, by selecting *Octomeria* as an outgroup in their analyses the authors cannot actually place *Sansonia* with confidence (because the underlying assumption that the ingroup is monophyletic would not be met). Unpublished DNA-based phylogenetic analyses place the *Sansonia* sequences sister to other *Octomeria* sequences (Karremans, in prep.). *Sansonia species* are creeping plants with short ramicauls and tubular flowers with a synsepal and two pollinia, characteristics that are

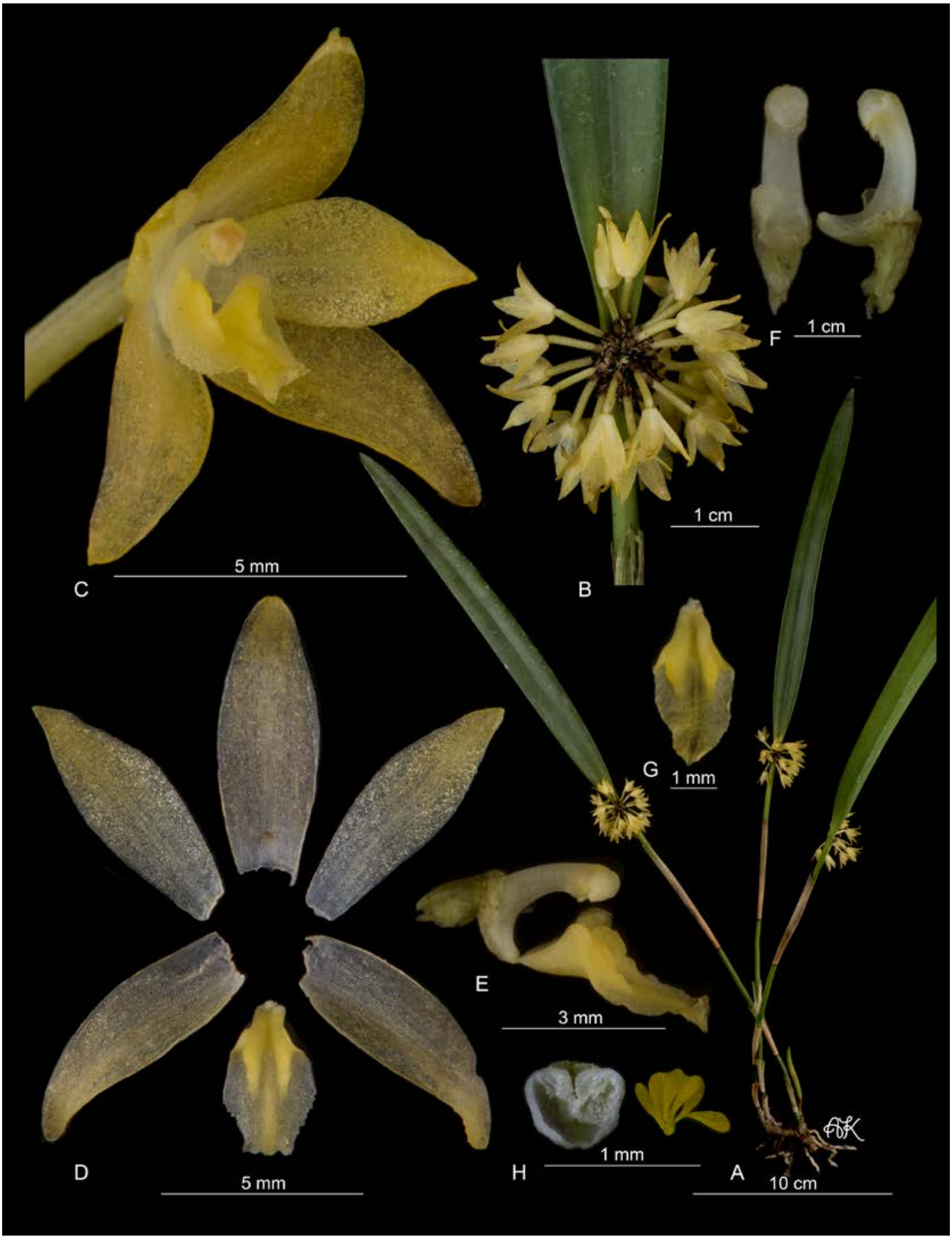


Figure 6. Lankester Composite Dissection Plate (LCDP) of *Octomeria valerioi* Ames & C.Schweinf. A. Habit. B. Inflorescence. C. Flower. D. Dissected perianth. E. Column and lip, lateral view. F. Column in ventral and lateral view. G. Flattened lip. H. Pollinia and anther cap. Photographs by A. Karremans based on *Bogarín 3017* (JBL-spirit).

generally found in derived groups of Pleurothallidinae. The genus is clearly different from *Pleurothallis* and *Octomeria*; nevertheless it is important to obtain DNA data from additional specimens of this genus to confirm its position.

Affinity *Phloeophila*

Genus *Phloeophila* Hoehne & Schltr. is the oldest name applicable to this group of species. It was recently reconsidered by Pridgeon and Chase (2001) on the basis of the molecular phylogeny published by Pridgeon *et al.* (2001). The study showed that the species of *Phloeophila* (in a strict sense) formed a unique clade together with species previously placed in *Luerella* Braas and *Ophidion* Luer and not closely related to *Pleurothallis*. The three genera were combined into a broadly circumscribed *Phloeophila* even though the species of all three had little morphological common ground. In a phylogenetic analysis of Brazilian Pleurothallidinae, Chiron *et al.* (2012) found accessions labeled *Phloeophila nummularia* (Rchb.f.) Garay, the type species of *Phloeophila*, embedded within *Pabstiella*. Nevertheless, this is a mistake that has been rectified (Chiron *et al.* 2016). In an independent analysis, Stenzel (2004) found Cuban accessions of *P. nummularia* to group with the other *Phloeophila* species as expected.

Morphologically, *Phloeophila* s.l. species are virtually undiagnosable. In fact, one knows that a species belongs to *Phloeophila* s.l. basically because it can be assigned on morphological grounds to either *Luerella*, *Ophidion* or *Phloeophila* s.s. From published and unpublished DNA data it is clear that even though species of all three genera form a single clade, each of them could also be monophyletic on their own. In fact, the DNA evidence also gives an explanation for the highly dissimilar morphologies as each of the three clades lies on long, highly divergent branches. The only reason this *Phloeophila* in its broad circumscription has remained a single genus is probably species number. If split up, *Luerella* would be monospecific, *Ophidion* would have six species, and *Phloeophila* s.s. only two. *Phloeophila* s.l. has nine species at this time (Chiron *et al.* 2016). Without doubt, if each genus had dozens or hundreds of species they would have been kept separate.

Affinity *Pleurothallis*

Three genera with different taxonomic histories belong within this affinity: *Pabstiella* Brieger & Senghas (Fig. 7), *Pleurothallis* R.Br. (Fig. 8), and *Stelis* Sw.; each of them is discussed separately.

In the DNA-based phylogenetic analysis of Pleurothallidiane by Pridgeon *et al.* (2001), *Pleurothallis mentosa* Cogn., *P. mirabilis* Schltr. and *P. tripterantha* Rchb.f. were found to form an isolated clade sister to *Pleurothallis* and *Stelis*. The authors published genus *Anthereon* Pridgeon & M.W.Chase to give generic recognition to the particular clade. However, the name is superfluous (and illegitimate) because *Pabstiella* had already been proposed for one of those species, *P. mirabilis*. *Gyalanthos* Szlach. & Marg., published almost simultaneously, was also based on *P. mirabilis,* and therefore it too is illegitimate. Only a handful of species were initially assigned to *Pabstiella* (Pridgeon 2005), but several dozen more species have since been recognized as belonging to the genus (Luer 2006, 2007), especially Brazilian species mistakenly believed to belong in *Specklinia* and *Stelis* s.l. The genus *Ronaldella* Luer was proposed to accommodate *Pleurothallis aryter* Luer and *P. determannii* Luer; DNA data placed them within *Pabstiella* (Karremans *et al.* 2013a).

Pleurothallis at some time or another included the species from the vast majority of other genera in this subtribe; notable exceptions are *Lepanthes*, *Masdevallia, Stelis*, and *Octomeria*. Luer (1986c) published a comprehensive subgeneric classification of *Pleurothallis*. Pridgeon and Chase (2001) clearly demonstrated the polyphyly of the genus and proposed a narrower circumscription of *Pleurothallis* by recognizing or broadening the genera *Acianthera*, *Anathallis*, *Andinia*, *Pabstiella*, *Phloeophila*, *Specklinia,* and *Stelis*. Luer (2004, 2006, 2007) followed with an even narrower concept of *Pleurothallis* by segregating the genera *Ancipitia* (Luer) Luer, *Elongatia* (Luer) Luer, *Lindleyalis* Luer, *Loddigesia* Luer (= *Lalexia* Luer), *Mixis* Luer, *Orbis* Luer, and *Tigivesta* Luer, while recognizing the previously proposed *Acronia* C.Presl, *Colombiana* Ospina, *Rhynchopera* Klotzsch and *Talpinaria* H.Karst. The recognition of these genera reduces *Pleurothallis* s.s. to less than a couple of hundred species, and the proposal has found little support by other authors.

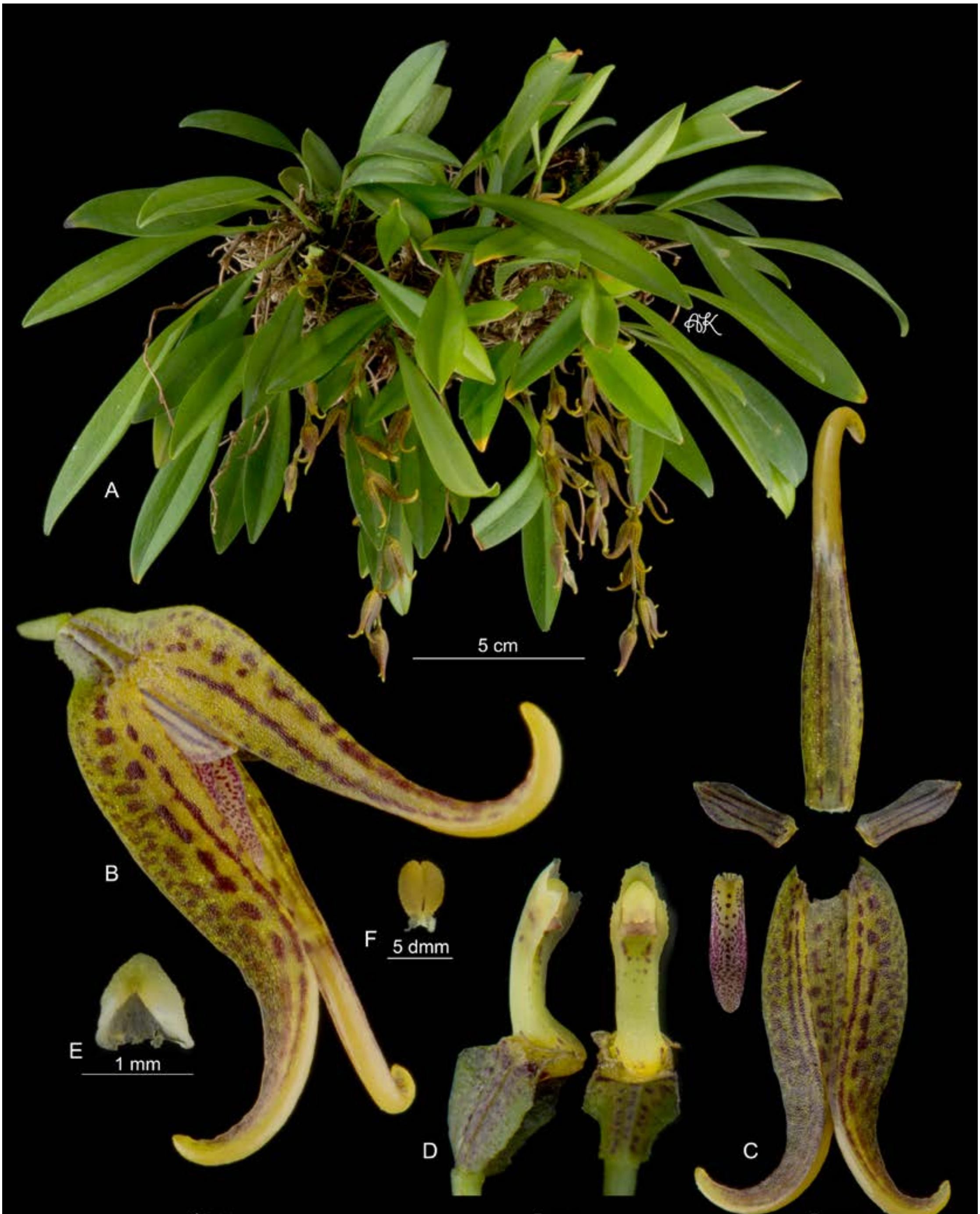


Figure 7. Lankester Composite Dissection Plate (LCDP) of *Pabstiella tripterantha* (Rchb.f.) F.Barros. A. Habit. B. Flower. C. Dissected perianth. D. Column in ventral and lateral view. E. Anther cap. F. Pollinarium. Photographs by A. Karremans and Jazmín Alomía based on *Bogarín 5512* (JBL-spirit).

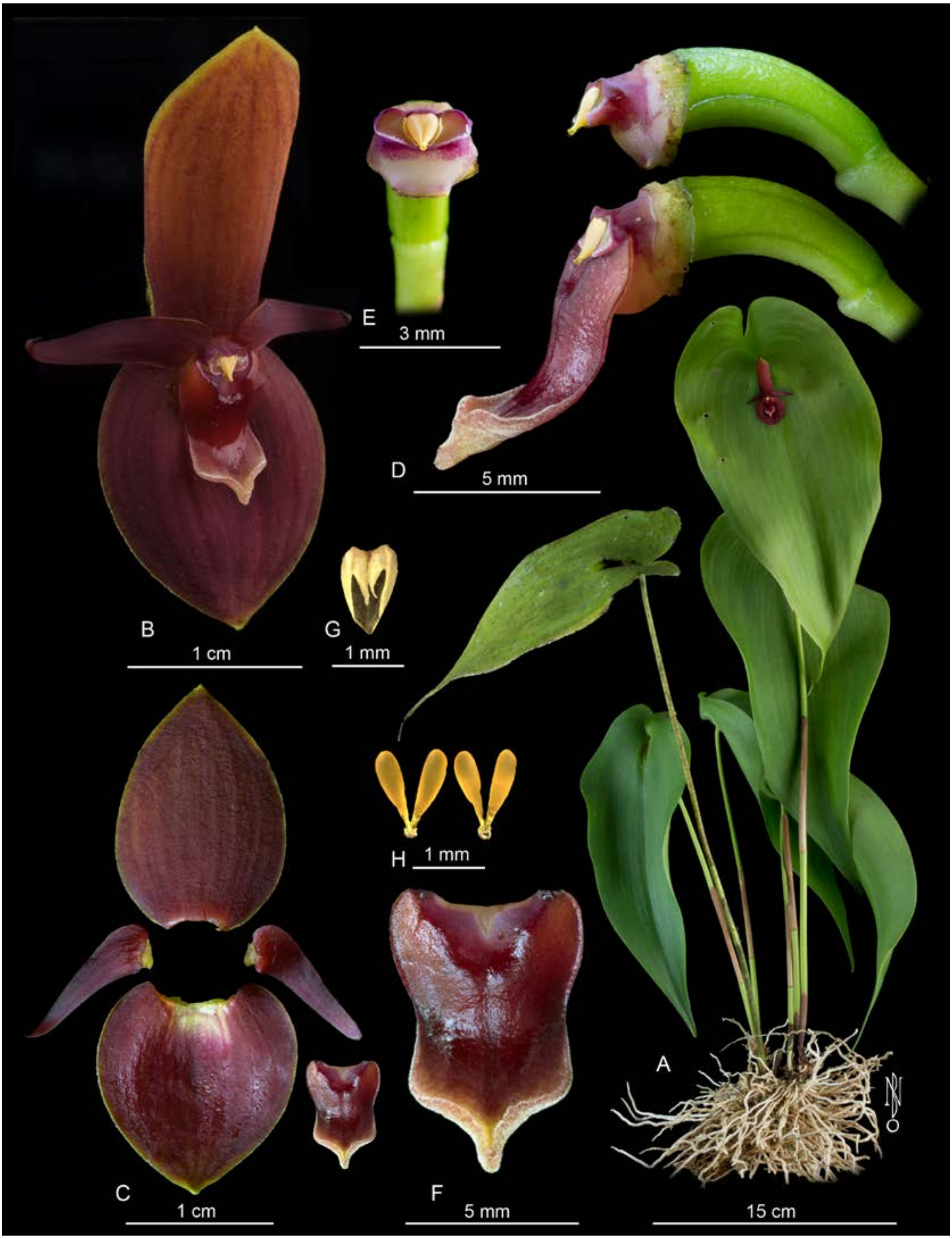


FIGURE 8. Lankester Composite Dissection Plate (LCDP) of *Pleurothallis cardiothallis* Rchb.f. A. Habit. B. Flower. C. Dissected perianth. D. Column and lip, lateral view. E. Column in ventral view. F. Lip. G. Anther cap. H. Pollinaria. Photographs by Noelia Belfort Oconitrillo and A. Karremans based on *Karremans 6580* (JBL-spirit).

Morphologically, the lines that separate *Ancipitia* from *Colombiana*, and *Acronia* from *Pleurothallis* s.s., are blurred, and *Elongatia* is undiagnosable. The non-monophyly of these genera is supported by published and unpublished DNA data (Karremans *et al.* 2013a, Wilson *et al.* 2013). Even though some of the other genera may indeed represent natural groups, the morphological features used to distinguish them are too plastic, and genetic distance seems to be low as well.

Species of *Stelis* had been traditionally easy to distinguish from species of other genera, and the genus had, until relatively recently, not been subjected to major taxonomic changes with the exception of a reduction proposed by Garay (1979). Pridgeon *et al.* (2001) proposed a broader circumscription of *Stelis* with the inclusion of several subgenera of *Pleurothallis* [i.e., *Crocodeilanthe* (Rchb.f. & Warsz.) Luer, *Dracontia* Luer, *Effusia* Luer, *Elongatia* Luer, *Mystax* Luer, *Physosiphon* (Lindl.) Luer, *Physothallis* (Garay) Luer, *Pseudostelis* (Schltr.) Luer, and *Unciferia* (Luer) Luer], as well as the smaller genera *Condylago* Luer and *Salpistele* Dressler. Luer (2002b) shed doubt on the accuracy of the DNA data and continued using the narrow circumscription of the genus (Luer 2009). Several authors would afterwards recognize some genera segregated from *Stelis* s.l., including *Condylago*, *Crocodeilanthe*, *Dracontia*, *Effusiella*, *Elongatia*, *Lomax*, *Niphantha*, *Physosiphon*, *Physothallis* and *Unciferia*, whereas other authors preferred to keep them within *Pleurothallis*.

The broader phylogenetic studies by Solano-Gómez (2005) and Karremans *et al.* (2013a) clearly showed that species of the above mentioned genera are all indeed more closely related to *Stelis* s.s. than to *Pleurothallis* s.s. Their analyses also demonstrated that genera such as *Effusiella* and *Elongatia* (Luer) Luer are polyphyletic and cannot be recognized as currently defined, and that genera such as *Crocodeilanthe*, *Dracontia*, *Physothallis*, *Salpistele* and *Unciferia,* although natural groups, are paraphyletic and deeply embedded with *Stelis* s.l. It is clear that there are some well-defined clades within *Stelis* s.l. and that those could eventually be recognized as distinct genera; however, for the time being it is impossible to tell which species should be assigned to what clade with any confidence. It is more advisable to maintain a monophyletic *Stelis* s.l. rather than recognize several polyphyletic and paraphyletic small genera.

Finally, it is important to mention that the genera *Elongatia* and *Lalexia* Luer (=*Loddigesia* Luer, *nom. illeg.*) should definitely be excluded from the synonymy of *Stelis* as DNA data have amply proven that their type species, *Pleurothallis restrepioides* Lindl. and *Pleurothallis quadrifida* (Lex) Lindl., respectively, are closer to the *Pleurothallis* than to *Stelis* (Karremans *et al.* 2013a, Wilson *et al.* 2013).

Affinity *Restrepia*

The eight genera accepted in this affinity -- *Barbosella* Schltr., *Chamelophyton* Garay, *Dresslerella* Luer, *Echinosepala* Pridgeon & M.W.Chase, *Myoxanthus* Poepp. & Endl. (Fig. 9), *Pleurothallopsis* Porto & Brade, *Restrepia* Kunth and *Restrepiella* Garay & Dunst. --have undergone less drastic changes in the last few decades. With the exception of *Echinosepala*, they were recognized as distinct when the first molecular phylogeny of the pleurothallids by Pridgeon *et al.* (2001) was published (Luer 1986a). In fact, the changes suggested by the authors of this particular group of genera were relatively few. Basically three changes were made: 1) placing *Barbrodia* Luer, embedded within *Barbosella* in the analyses, under the synonymy of the latter; 2) reducing *Restrepiopsis* Luer under the synonymy of its sister genus *Pleurothallopsis*; 3) creating a new genus, *Echinosepala*, for a group of species previously assigned to *Myoxanthus* but not part of that clade. Unpublished DNA data from a broader set of species belonging to each of the genera mentioned confirm that as currently defined they are all monophyletic. Contrary to what was found by Pridgeon *et al.* (2001), *Myoxanthus* and *Echinosepala* are apparently sister genera. There would be little to gain by lumping the two genera as they are perfectly diagnosable and informative. To my knowledge, no DNA data (published or unpublished) is available of the monospecific genus *Chamelophyton* to confirm its exact phylogenetic relationships. However, based on the similar basal lobes of the lip, slender wing-less column and multiple pollinia, it seems to be related to species of *Pleurothallopsis*. The single species belonging to this genus is rarely found in Venezuela and Guayana.

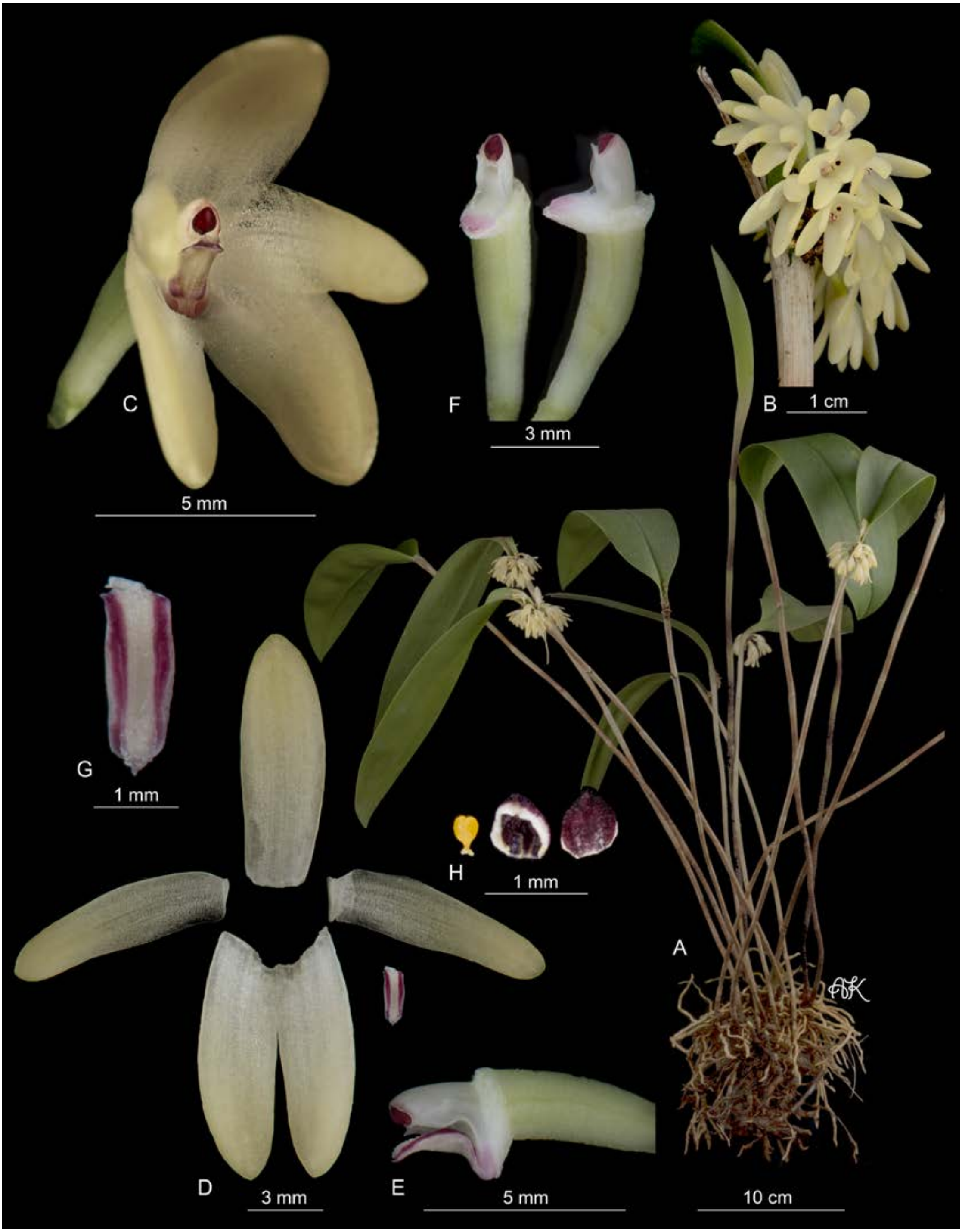


FIGURE 9. Lankester Composite Dissection Plate (LCDP) of *Myoxanthus octomeriae* (Schltr.) Luer. A. Habit. B. Inflorescence. C. Flower. D. Dissected perianth. E. Column and lip, lateral view. F. Column in ventral and lateral view. G. Lip. H. Pollinia and anther cap. Photographs by A. Karremans and Gustavo Rojas Alvarado based on *Karremans 5693* (JBL-spirit).

Affinity *Specklinia*

This affinity is currently made up of seven accepted genera: *Andinia* (Luer) Luer, *Dryadella* Luer, *Muscarella* Luer, *Platystele* Schltr. (Fig. 10), *Scaphosepalum* Pfitzer (Fig. 11), *Specklinia* Lindl., and *Teagueia* (Luer) Luer.

On one hand, *Dryadella*, *Platystele,* and *Scaphosepalum* were all treated as distinct, well-recognized genera when Luer published his first monograph of Pleurothallidinae (Luer 1986a). The author would later segregate *Teagueia* from *Platystele* (1991), and the four genera would be generally accepted when Pridgeon *et al.* (2001) published their molecular phylogeny of Pleurothallidinae. On the other hand, the oldest generic name applicable to this affinity, *Specklinia*, had not been used for about 150 years, until it was "resurrected" from the synonymy of *Pleurothallis* by Pridgeon & Chase (2001). DNA data clearly showed that *Specklinia* was sister to a clade composed of *Platystele* and *Scaphosepalum*; sister to all of them was *Dryadella*. Luer (2002b) did not recognize *Specklinia* initially but would later make many transfers expanding the circumscription of the genus (Luer 2004) and finally would reduce it to a handful of species by recognizing several new segregate genera (Luer 2006). The DNA data published by Pridgeon *et al.* (2001) showed that *Acostaea* Schltr., *Pseudoctomeria* Kraenzl., *Empusella* Luer, *Muscarella* Luer, *Tribulago* Luer, and *Sarcinula* Luer all belonged within their newly circumscribed *Specklinia*. A phylogenetic analysis including a broader sampling of *Specklinia* species confirmed the previous findings, but also places the genera *Cucumeria* Luer, *Gerardoa* Luer, and *Sylphia* Luer (on the basis of DNA data), and *Areldia* Luer and *Tridelta* Luer, (on the basis of morphology) within *Specklinia* (Karremans 2015, Karremans *et al.*, in press).

The new study also shows that *Muscarella* Luer should be excluded from *Specklinia* if it is to be maintained as monophyletic, because *Muscarella* is sister to the clade that includes *Platystele*, *Scaphosepalum,* and *Specklinia*. It also places *Incaea* Luer (a genus previously believed to belong in *Phloeophila)* within *Dryadella*, whereas *Rubellia* (Luer) Luer, previously unplaced, is sister to *Platystele*. The latter relationships are supported by overall morphology and do seem reasonable. The position of *Teagueia* is not completely resolved. DNA data from a single accession places it in the *Platystele-Scaphosepalum* clade, but it is unclear to which genus it is most closely related. A broader sampling *Teagueia* species will allow for a better understanding of its relationships.

Finally, the concept of genus *Andinia* that is accepted here follows that which was proposed by Wilson *et al.* (in press). Many of its species had traditionally been placed in *Lepanthes*. The first step towards recognizing them as distinct was made by Luer (1986a), who transferred *L. dielsii* Mansf. and *L. pensilis* Schltr. to *Salpistele* Dressler. The two misfits were almost immediately given subgeneric recognition (Luer 1991), followed by generic status under the name *Andina* (Luer 2000a). Pridgeon and Chase (2001) broadened *Andinia* to include 10 species, the two from *Salpistele* together with several previously placed in *Pleurothallis*, on the basis of DNA data. Wilson *et al.* (in press) expanded *Andinia* even more on the basis of DNA data and morphological similarities. The authors showed that the *Lepanthes nummularia* Rchb.f. group, which had long been placed in *Lepanthes*, and was recently also given generic recognition under the illegitimate generic name *Brachycladium* (Luer) Luer and a few replacement genera, is phylogenetically allied to the species already placed in *Andinia*. Wilson *et al.* (in press) place *Andinia* in the *Specklinia* rather than the *Pleurothallis* clade where it has been found in other studies. The issue will undoubtedly need to be re-evaluated in the future with more data at hand.

Uncertain affinities

Andreettaea Luer, Selbyana 2: 183. 1978.

A single species of *Andreettaea* is known. It is apparently without close relatives. Luer (1986c) believed it to be allied with species of *Pleurothallis* subgen. *Specklinia* because of the abbreviated ramicaul. Nevertheless, we know now that this feature has appeared many times independently in Pleurothallidinae, and the species then assigned to *P.* subgen. *Specklinia* are now placed in many different genera. The non-resupinate flowers with fused sepals, acuminate petals, conspicuously lobbed lip, and pollinia with caudicles surely exclude it from genus *Specklinia*. It might belong in the *Phloeophila* affinity. DNA data will probably be needed to place *Andreettaea* among its relatives; it is clearly not a synonym of *Pleurothallis*

Figure 10. Lankester Composite Dissection Plate (LCDP) of *Platystele oxyglossa* (Schltr.) Garay. A. Habit. B. Flower. C. Dissected perianth. D. Column and lip, lateral view. E. Column and lip in frontal view. F. Pollinia and anther cap. Photographs by A. Karremans based on *Karremans 7074* (JBL-spirit).

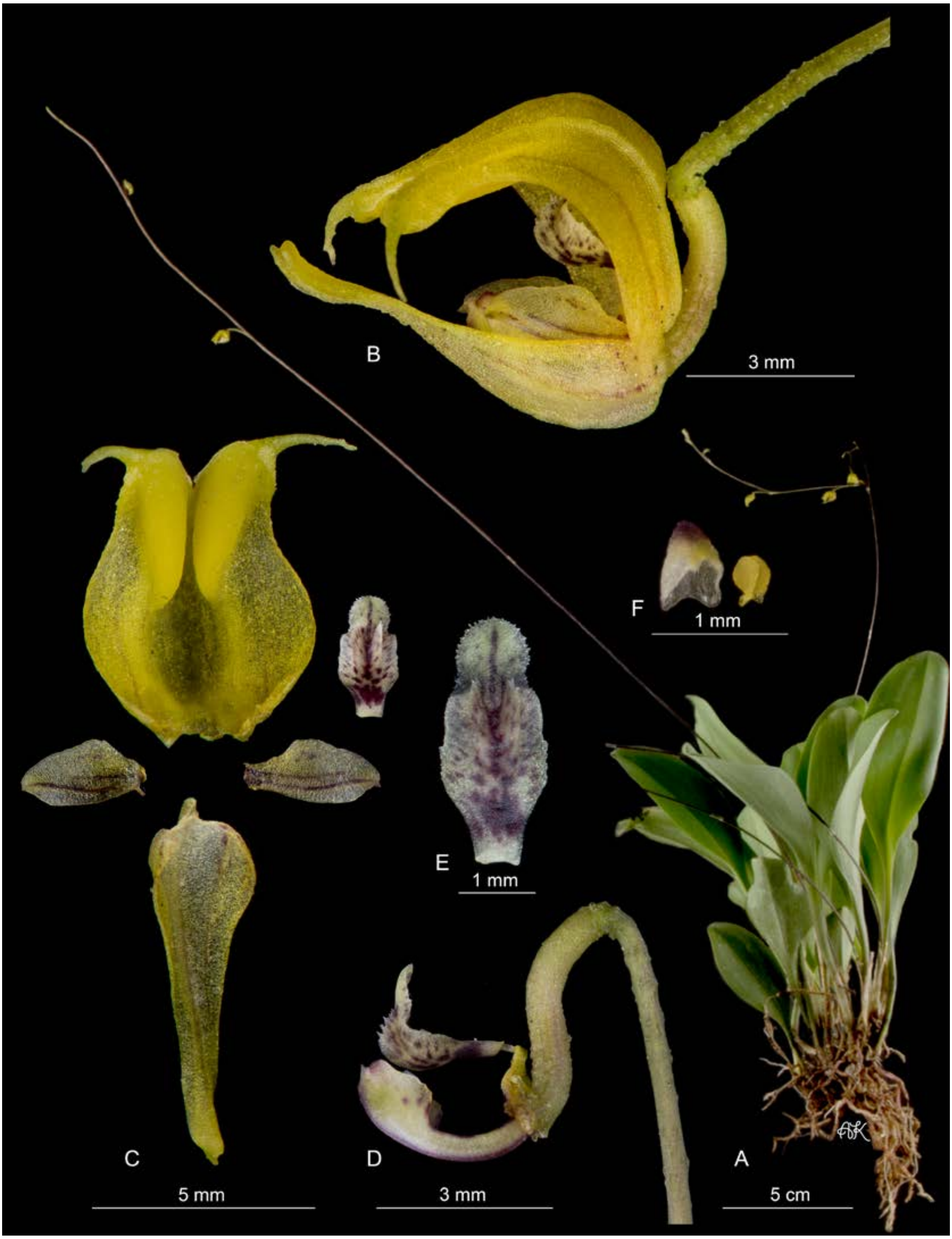


FIGURE 11. Lankester Composite Dissection Plate (LCDP) of *Scaphosepalum verrucosum* (Rchb.f.) Pfitzer. A. Habit. B. Flower. C. Dissected perianth. D. Column and lip, lateral view. E. Lip. F. Pollinia and anther cap. Photographs by AK based on *JBL-990078* (JBL-spirit).

Madisonia. Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 258. 2004.

When describing *Madisonia*, Luer (2006) did not mention any closely related species or genera. The only known species is distinguished by a creeping rhizome and abbreviated ramicauls (both covered by ciliate sheaths); sessile, subcircular leaves; an elongated peduncle; and a single flower with a conspicuous mentum. *Madisonia* is indeed unique for this combination of features, and there are only two genera that would have somewhat similar species, *Anathallis* and *Pabstiella*. *Anathallis spiculifera* (Lindl.) Luer shares the long repent habit, abbreviated ramicaul, elongated peduncle, and three-lobed lip. Also similar is *Pabstiella brasilica* Luer & Toscano, which shares the repent habit, abbreviated ramicaul, elliptical leaves, elongate peduncle, single flowers, connate lateral sepals, and three-lobed lip. It is hard to believe these three species belong to different genera, but it is too soon to say if they are indeed sister species and if they should be assigned to either *Anathallis*, *Madisonia* or *Pabstiella*. Luer and Toscano (2012) clearly had the same issue with these species; when describing *Pabstiella brasilica* their comparative description was made with a species they believed belongs to a different genus, *Anathallis spiculifera*. This is highly unusual because one normally compares a species with its closest relative, which of course cannot be a species from an unrelated genus. DNA data will surely be helpful in placing these three species in the future.

Conclusions. Whether more and narrowly defined or less and broadly circumscribed genera should be recognized is constantly being debated among botanists and hobbyists alike. Pleurothallidinae with 160 proposed generic names today is probably among the most debated groups in that sense. As circumscribed in *Genera Orchidacearum* (GO), the subtribe included only 37 accepted genera (Pridgeon 2005). Considering there are currently more than 5100 accepted species in the subtribe, Pleurothallidinae would average about 138 species per genus. That is, as defined in GO, more than double the average number of species per genus in Laeliinae, five times that average in Oncidiinae, and more than ten times the average in Zygopetalinae. There are possibly two basic explanations for this difference -- either speciation within Pleurothallidinae has been more explosive than in the others, or there is some sort of size-related bias. The answer is most likely both. Givnish *et al.* (2015) showed that Pleurothallidinae has one of the highest species diversification rates in the Orchidaceae. With just above 10 My of age, the subtribe is about one third younger than Laeliinae and Oncidiinae and has more than double the number of species. This means, in general terms, not only that speciation has been faster in Pleurothallidinae as compared to Laeliinae and Oncidiinae, but also that the genera belonging to the latter have had more evolutionary time to establish and differentiate. On the other hand, size does matter. We have a harder time finding and accepting morphological differences as significant in smaller organisms, especially if those organisms come in large numbers. It is in that sense to be expected that genera and species in Pleurothallidinae are more broadly circumscribed than in other orchid groups. Nevertheless, we should take care not to mask relevant speciation events and lineage diversification by being too inclusive. It is our main task to document and describe biodiversity even when that means having more names than one can remember. We are after all dealing with a subtribe that includes about 20% of all known orchids.

In this update of the phylogenetic relationships within Pleurothallidinae, 44 genera with 5114 species are recognized. However, it is clear that much more work needs to be done in the future to fully resolve and understand the systematic placement of all lineages within this megadiverse subtribe. The generic circumscriptions of *Acianthera*, *Masdevallia*, *Phloeophila*, *Pleurothallis*, *Specklinia,* and *Stelis* are, irrespective of species numbers, extremely inclusive and might not be as informative as we would like. Nevertheless, they are grossly monophyletic, and thus it is a good starting point for their reconsideration with additional evidence. It is important to take into consideration that dozens of Pleurothallidinae are described each year and that whole lineages might still need recognition. The placement of species in discrete, manageable groups makes it easier to study them in detail and normally results in the recognition of additional species. The subtribe will surely grow by several hundred species in the coming decades as more literature becomes available. The growth in species numbers will certainly be accompanied by the need for more generic and subgeneric taxa. As Lindley (1842) eloquently put it when discussing the pleurothallids, "they have moreover been insufficiently classified, in consequence of which the difficulty of their determination has been much increased."

Acknowledgements. I wish to thank Franco Pupulin for encouraging the preparation of this manuscript. Alec Pridgeon and an anonymous reviewer kindly improved an earlier version of this manuscript with their comments.

LITERATURE CITED

Abele, A. D. (2007). Phylogeny of the genus *Masdevallia* Ruiz et Pav. based on morphological and molecular data. Ph.D. thesis, University of Hamburg, Germany, Dec 2007.

Bogarín, D., Karremans, A. P. & Pupulin, F. (2008). New species and records of Orchidaceae from Costa Rica. *Lankesteriana* 8(2): 53-74.

Bogarín, D., Karremans, A. P., Rincón, R. & Gravendeel, B. (2013). A new *Specklinia* (Orchidaceae: Pleurothallidinae) from Costa Rica and Panama. *Phytotaxa* 115(2): 31-41.

Borba, E. L., Barbosa, A. R., Cabral de Melo, M., Loureiro, S. & Ornellas de Oliveira, H. (2011). Mating systems in the Pleurothallidinae (Orchidaceae): evolutionary and systematic Implications. *Lankesteriana* 11(3): 207-221.

Chiron, G. R., Guiard J. & van den Berg, C. (2012) Phylogenetic relationships in Brazilian *Pleurothallis* sensu lato (Pleurothallidinae, Orchidaceae): evidence from nuclear ITS rDNA sequences. *Phytotaxa* 46: 34–58.

Chiron, G. R., Karremans, A. P. & van den Berg, C. (2016) Nomenclatural notes in the Pleurothallidinae (Orchidaceae): *Phloeophila*. *Phytotaxa* 270: 56–62.

Endara, L., Williams, N. H. & Whitten, W. M. (2011). Filogenia molecular preliminar de *Scaphosepalum* (Orchidaceae: Pleurothallidinae). *Lankesteriana* 11(3): 245–252.

Forster, W. (2007). Estudo taxonômico das espécies com folhas planas a conduplicadas do gênero *Octomeria* R.Br. (Orchidaceae). Doctoral Thesis. Universidade de São Paulo, São Paulo.

Garay, L.A. (1979) Systematics of the genus *Stelis* Sw. *Botanical Museum Leaflets* 27: 167–259.

Givnish, T. J., Spalink, D., Ames, M, Lyon, S. P., Hunter, S. J., Zuluaga, A., Iles, W. J. D, Clements, M. A., Arroyo, M. T. K., Leebens-Mack, J., Endara, L, Kriebel. R., Neubig, K. M., Whitten, W. M., Williams, N. H. & Cameron, K. M. (2015). Orchid phylogenomics and multiple drivers of their extraordinary diversification. *Proceedings Royal Societ B* 282: 20151553.

Karremans, A. P. (2010) Phylogenetics of *Stelis* (Orchidaceae: Pleurothallidinae) and closely related genera, based on molecular data, morphological characteristics and geographical distribution in the Central American and Andean Cordilleras. MSc Thesis, Plant Sciences Group and Biosystematics Group, Wageningen University.

Karremans, A. P. (2014). *Lankesteriana*, a new genus in the Pleurothallidinae (Orchidaceae). *Lankesteriana* 13(3): 319-332.

Karremans, A. P. (2015). Systematic, phylogenetic and pollination studies of *Specklinia* (Orchidaceae). Dissertation Thesis, Leiden University, The Netherlands.

Karremans, A. P. & Rincón-González, M. (2015). Nomenclatural notes in the Pleurothallidinae (Orchidaceae): *Apoda-prorepentia*. *Phytotaxa* 238(2): 174-182.

Karremans, A. P., Albertazzi, F. J., Bakker, F. T., Eurlings, M. C. M., Pridgeon, A., Pupulin, F. & Gravendeel, B. (in press) Phylogenetic reassessment of *Specklinia* and its allied genera in the Pleurothallidinae (Orchidaceae). *Phytotaxa*.

Karremans, A. P., Bakker, F. T., Pupulin, F., Solano-Gómez, R. & Smulders, M. J. M. (2013a). Phylogenetics of *Stelis* and closely related genera (Orchidaceae: Pleurothallidinae). *Plant Systematics and Evolution* 29(1): 69-86.

Karremans, A. P., Pupulin, F. & Gravendeel, B. (2013b). Taxonomy, molecular phylogenetics, reproductive isolation, and niche differentiation of the *Specklinia endotrachys* species complex (Orchidaceae: Pleurothallidinae). *Lankesteriana* 13(1-2): 132-133.

Lindley, J. (1842). Pleurothallis. *Edwards's Botanical Register* 28: 67-84.

Lindley, J. (1859). Folia Orchidacea. *Pleurothallis*.

Luer, C. A. (1986a). Icones Pleurothallidinarum I. Systematics of the Pleurothallidinae (Orchidaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* 15.

Luer, C. A. (1986c). Icones Pleurothallidinarum III. Systematics of the genus *Pleurothallis* (Orchidaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* 20.

Luer C. A. (1991). Icones Pleurothallidinarum VIII Systematics of *Lepanthopsis*, *Octomeria* Subgenus *Pleurothallopsis*, *Restrepiella*, *Restrepiopsis*, *Salpistele*, and *Teagueia* (Orchidaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* 39.

Luer, C. A. (2000a). Icones Pleurothallidinarum. XX. Systematics of *Jostia*, *Andinia*, *Barbosella*, *Barbrodia*, *Pleurothallis* subgen. *Antilla*, subgen. *Effusia*, subgen. *Restrepioidia*. Addenda to *Lepanthes*, *Masdevallia*, *Platystele*, *Pleurothallis*, *Restrepiopsis*, *Scaphosepalum*, and *Teagueia*. *Monographs in Systematic Botany from the Missouri Botanical Garden* 79.

Luer, C. A. (2000b). Icones pleurothallidinarum. XXI. Systematics of *Masdevallia* part two. *Monographs in Systematic Botany from the Missouri Botanical Garden* 82.

Luer, C. A. (2002a). Icones Pleurothallidinarum XXIV: A first century of new species of *Stelis* of ecuador, part one. *Monographs in Systematic Botany from the Missouri Botanical Garden* 88.

Luer, C. A. (2002b). A Systematic Method of Classification of the Pleurothallidinae Versus a Strictly Phylogenetic Method. *Selbyana* 23(1): 57-110.

Luer, C. A. (2004). Icones Pleurothallidinarum XXVI. *Pleurothallis* subgenus *Acianthera* and three allied subgenera. A second century of new species of *Stelis* of Ecuador. *Epibator*, *Ophidion*, *Zootrophion*. *Monographs in Systematic Botany from the Missouri Botanical Garden* 95.

Luer, C. A. (2005). Icones Pleurothallidinarum XXVII. *Dryadella* and *Acronia* section *Macrophyllae-Fasciculatae*. *Monographs in Systematic Botany from the Missouri Botanical Garden* 103.

Luer, C. A. (2006). Icones Pleurothallidinarum XXVIII. Reconsideration of *Masdevallia*, and the Systematics of *Specklinia* and vegetatively similar genera (Orchidaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* 105.

Luer, C. A. (2007). Icones Pleurothallidinarum XXIX. A third century of *Stelis* of Ecuador and Systematics of *Apoda-Prorepentia* and Systematics of Miscellaneous small genera, addenda: new genera, species, and combinations (Orchidaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* 112.

Luer, C. A. (2009). lcones Pleurothallidinarum XXX. *Lepanthes* of Jamaica and Systematics of *Stelis*, *Stelis* of Ecuador, part four and addenda: systematic of *Masdevallia*, new species of *Lepanthes* from Ecuador, and miscellaneous new combinations. *Monographs in Systematic Botany from the Missouri Botanical Garden* 115.

Luer, C. A. & Toscano de Brito, A. L. V. (2012). Miscellaneous new species in the Pleurothallidinae (Orchidaceae) from Brazil. *Harvard Papers in Botany* 17(2): 307-315.

Matuszkiewicz, A. & Tukallo, P. (2006). Infrageneric reclassification and phylogenetic inference in the genus *Masdevallia* Ruiz & Pav. – preliminary results of nrDNA based analysis. *Biodiversity Research and Conservation* 3-4: 213-219

Meyer, G. & Cameron, K. M. (2009). A preliminary phylogenetic study of *Dracula* (Pleurothallidinae, Epidendroideae, Orchidaceae) based on plastid *matK* sequence data. pp. 100-114. In Pridgeon, A. M & Suárez, J. P. (eds.*) Proceedings of the Second Scientific Conference on Andean Orchids*. Universidad Técnica Particular de Loja, Loja, Ecuador.

McDaniel, J. & Cameron, K. M. (2015). Phylogenetic utility of genotyping by sequencing (GBS) at the species level: an example from flowering plants (*Porroglossum*: Orchidaceae). Abstract - Botany 2015, Botanical Society of America Annual Meeting, Edmonton, Canada.

Neyland, R., Urbatsch, L. E. & Pridgeon, A. M. (1995). A phylogenetic analysis of subtribe Pleurothallidinae (Orchidaceae). *Botanical Journal of the Linnaean Society* 117: 13-28.

Pessoa, E., Valsko, J. J., Vasconcelos, S., Benko-Isepon, A. M., & Alves, M. (2014). *Anathallis roseopapillosa* (Orchidaceae – Pleurothallidinae) from the Central Amazon Region. *Systematic Botany* 39(4): 1070-1075.

Pridgeon, A. M. & Chase, M. W. (2001). A phylogenetic reclassification of Pleurothallidinae (Orchidaceae). *Lindleyana* 16(4): 235-271.

Pridgeon, A. M., Solano-Gómez, R. & Chase, M. W. (2001). Phylogenetic relationships in Pleurothallidinae (Orchidaceae): combined evidence from nuclear and plastid DNA sequences. *American Journal of Botany* 88(12): 2286-2308.

Pridgeon, A. M. (2005). Subtribe Pleurothallidinae. In: A. M. Pridgeon, P. J. Cribb, M. W. Chase & F. N. Rasmussen (eds.), *Genera Orchidacearum. Volume 4 Epidendroideae (Part One)*. Pp. 319-422.

Solano-Gómez, R. (2003). *Acianthera unguicallosa* (L.O.Williams) Solano. Pl. 512. In: Hágsater, E. & Soto, M. A. (eds.). *Orchids of Mexico, parts 2 and 3. Icones Orchidacearum* 5 & 6: pl. 501–700.

Solano-Gómez, R. (2005). Inference of the phylogenetic relationships in *Stelis sensu lato* clade based upon morphology and sequences of the ITS region data sets. In Raynal-Roques, A., Roguenant, A. & Prat, D. (eds.) *Proceedings of the 18th World Orchid Conference*, March 2005, Dijon, France.

Solano-Gómez, R. (2015). A taxonomic synopsis of the Mexican species of *Acianthera* (Orchidaceae: Pleurothallidinae) including a new species. *Phytotaxa* 218(1): 93–60.

Stenzel, H. (2000). Pollen morphology of the subtribe Pleurothallidinae Lindl. (Orchidaceae). *Grana* 39: 108-125.

Stenzel, H. (2004). Systematics and evolution of the genus *Pleurothallis* R. Br. (Orchidaceae) in the Greater Antilles. Dissertation Thesis. Mathematisch-Naturwissenschaftlichen Fakultät I der Humboldt- Universität zu Berlin.

Stenzel, H. (2007). Orchidaceae II. Pleurothallidinae 1. Pp. 1-152 in W. Greunter & R. Rankin Rodríguez, eds. Flora de la Republica de Cuba. Fascicle 12(2). Gantner, Liechtenstein.

Wilson, M., Belle, C., Dang, A., Hannan, P., Kellogg, L., Kenyon, C., Low, H., Mochizuki, A., Nguyen, A., Sheade, N., Shan, L., Shum, A., Stayton, T., Volz, C., Vosburgh, B., Wellman, H. & Woolley, M. A. (2013). Preliminary phylogenetic analysis of *Pleurothallis sensu lato* based upon nuclear and plastid sequences. (Abstract of poster presented at 4th Scientific Conference on Andean Orchids, November 2012, Guayaquil, Ecuador.) *Lankesteriana* 13(1-2):139.

Wilson, M., Frank, G. S., Jost, L., Pridgeon, A. M., Vieira-Uribe, S., & Karremans, A. P. (in press). Phylogenetic analysis of *Andinia* (Orchidaceae: Pleurothallidinae) and a systematic re-circumscription of the genus. *Phytotaxa.*

LANKESTERIANA 16(2): 243–254. 2016.

doi: http://dx.doi.org/10.15517/lank.v16i2.26027

ACTIVE TECTONIC AND VOLCANIC MOUNTAIN BUILDING AS AGENTS OF RAPID ENVIRONMENTAL CHANGES AND INCREASED ORCHID DIVERSITY AND LONG-DISTANCE ORCHID DISPERSAL IN THE TROPICAL AMERICAS: OPPORTUNITIES AND CHALLENGES

Stephen H. Kirby

Scientist Emeritus, U.S. Geological Survey, Menlo Park, CA 94025, U.S.A.
Visiting Professor, Faculty of Sciences, Tohoku University, Sendai, Japan
Honorary Professor, Institute of Risk and Disaster Reduction, University College London, London WC1E 6BT, U.K.
Research Associate, Lankester Botanical Garden, University of Costa Rica
stevenlyle@icloud.com

Abstract. Tropical Latin America is a nexus of tectonic plates whose relative motions have led to rapid tectonic and volcanic mountain building in late Neogene time. Tropical mountain building, in turn, leads to highland "cloud forest" microclimates with increased rainfall, lower diurnal temperatures, and diverse microclimates. I have previously emphasized how the geologically recent growth of mountains has been localized in Central America and that this is likely a factor in the high diversity and endemism in those highlands. This paper will show that Andean uplift accelerated at ~15 Ma ago and ~5 Ma BP and continues to this day. This process evolved geographically among the cordilleras of the region. Givnish and others recently presented phylogenomic evidence that the diversity of many epiphytic orchids, including tribes found in the neotropics, also accelerated during this time interval. Phylogenetic investigations of tropical orchid pollinators have shown that acceleration in speciation in such pollinators as hummingbirds, orchid bees, and flies occurred over this same time frame, suggesting that geologically driven environmental changes may have acted in concert with changes in orchid biology to speed up orchid diversity in these highlands. I also review some of the long-distance dispersal processes of orchids in the tropical Americas. River systems draining the Colombian Andes discharge into the Caribbean Sea and current-driven log-raft drifts and air suspension during cyclonic storms transport plants and animals from east to the west. Lastly I emphasize the need for the more information on orchid floras and species distribution in this hotspot.

Key words: Andean orchids, mountain building and orchids, Neotropics, orchid diversity, orchid species distribution

Prologue. It is the author's pleasure to acknowledge the important contributions of Calaway Dodson to neotropical orchid biogeography and diversity and his encouragement of the author to do research on geological processes can be important contributors to orchid diversity. In 2003 he wrote: "Given the nature of the orchids subject to [biological factors] and the *changing geology and microclimates of Ecuador* [my italics], rapid change in the genetic nature of populations leading to explosive speciation is possible" (Dodson 2003).

Introduction. There are few places on this planet that are as geologically active as the nexus in the tropical Americas where there are five major tectonic plates, five microplates, seven triple junctions where three plates meet, and seven trenches where one plate dives under another, a process called *subduction* (Fig. 1). The relative motions of these plates and microplates lead to a rapidly changing environment of tectonic mountain building by compression and thrust faulting localized along major faults and volcanic mountain building where the positions of active arc volcanic centers and volcanic chains often change largely due to changes in the nature of the incoming plate and the geometry of subducting plate. These

Received 10 May 2016; accepted for publication 22 August 2016. First published online: 30 August 2016.

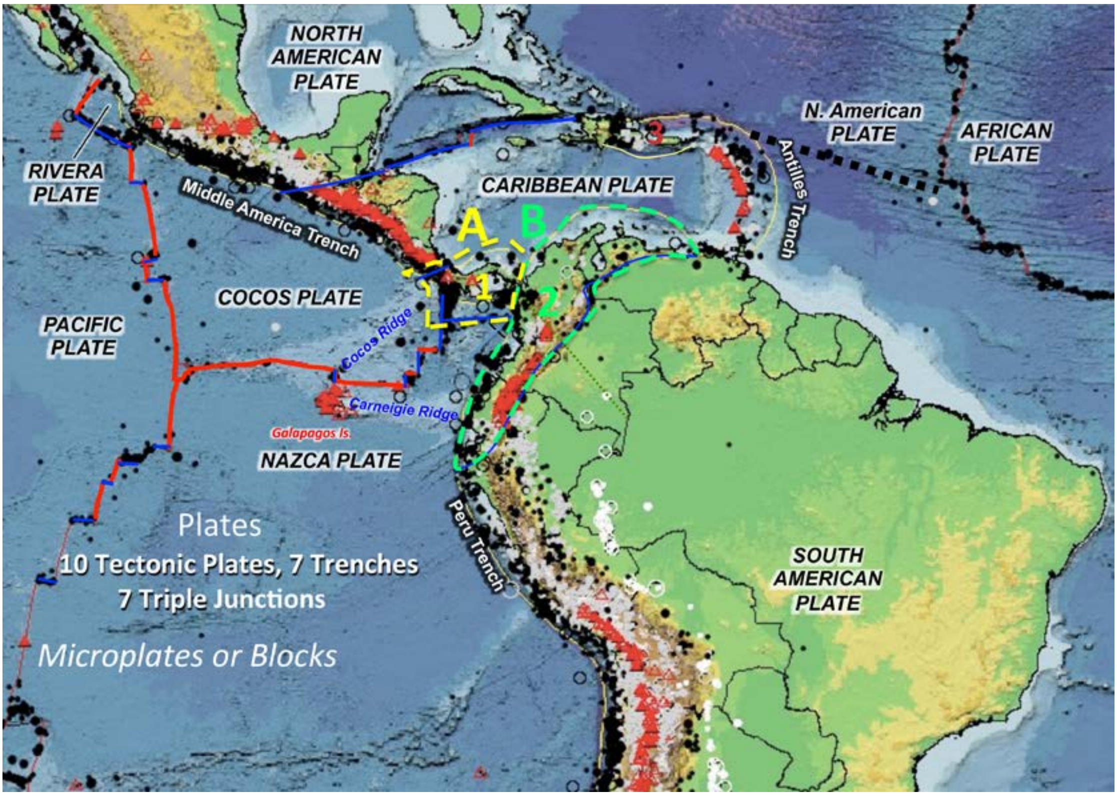


FIGURE 1. Map of neotropical orchid and tectonic diversity hotspots illustrating geologic-based environmental changes as possible drivers for orchid diversity. A) Costa Rica and Panama (Kirby, 2009) in the SE Cocos Plate and Panama/Coiba Block (marked by the yellow dashed line); B) Northern Andes Uplift: Ecuador, Colombia, and NW Venezuela (marked by the dashed green line) . Map created by the *This Dynamic Planet* online tools (Simkin et al., 2006). After a figure in Kirby (2009, with permission). Volcanoes: red triangles; black dots: Shallow earthquakes (<60 km depth); Gray dots: Deeper earthquakes; Red lines: ocean spreading centers; blue lines: transform or strike-slip plate boundaries; yellow lines: trenches where subduction begins.

changes in the neotropics have been documented using radiogenic isotopes and paleontology, palynology, and stratigraphic and other geologic and geophysical methods used both on land and under the ocean. Present-day relative plate motions are indicated by the distribution of earthquakes and GPS measurements. The development of a tectonic model for this region that draws upon these observations and is paralleled by increasingly precise dating of branch points in the orchid tree of life and those of several groups of orchid pollinators. One aim of this paper is to compare the geologic dates of these geological and biological events and discuss the insights that can be drawn from these comparisons. Lastly, the author describes the meteorological, hydrological, and oceanographic processes that likely govern the dispersal of neotropical orchid species and the enhanced sampling and taxonomic needed in Andean Colombian and eastern Panamanian forests to test these dispersal hypotheses.

As an aid to understanding the geological time scale, I provide Figure 2, that shows the Periods and Epochs of the Cenozoic Era in the last 66 Ma (a Ma is a mega *anunm*, or a million years).

Orchid species distributions in the tropics by country or major island. In Table 1, I attempt to summarize various estimates of the number of orchid species by tropical country or island for those areas that have among the highest species counts and degrees of endemism. This is a difficult task because of differences in forest access related to security

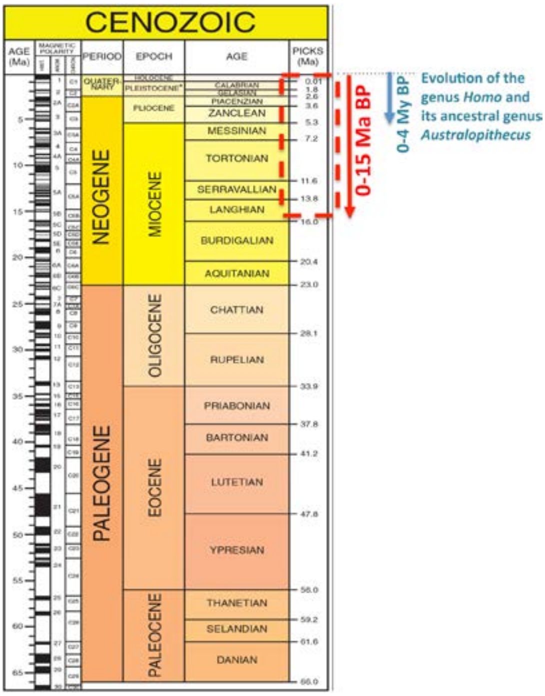


Figure 2. Tropical America was a very busy place in the last 15 million years, both geologically and biologically, linked by rapid environmental changes in highland micro-climates. Source: Geological Society of America Website.

issues and terrane ruggedness and isolation and in the degrees of collection and taxonomic effort. However, observers generally agree that the northern Andes (Ecuador and Colombia), SE Central America (Costa Rica and Panama) and the Island of New Guinea (West Papua, Irian Jayra, Indonesia and the main island part of Papua New Guinea) are the places where orchid species counts are large and the numbers of species new to science continue to be described. Allowing for big differences in sampling effort and taxonomic research effort, these localities have among the highest species counts and rates of endemism in the tropical world. It is notable that these areas also represent among the most tectonically complicated and active in the tropics and are also notable that they have prominent cordilleras and that these mountain ranges are young or have been rejuvenated by relatively recent tectonic uplift (middle Miocene (starting about ~12-15 Ma BP and accelerating at 4-6 Ma (Pliocene) (Fig. 2). Mountain building continues in these regions, as demonstrated by the high level of seismic activity, volcanic eruptions, and geodetic measurements (using GPS instruments) of crustal deformation. Dodson (2003) and Kirby (2007, 2011), Ackerman (2012, 2014) have described how these geological processes can lead to rapid environmental changes, geographic isolation into microclimates, and mutagenesis, all-important factors in speciation. In the next section I explain the special plate tectonic settings that led to the geologically young, intense, and ongoing mountain building in these regions.

Tectonics of the Costa Rica-Panama/Colombia-Ecuador Nexus. Geological factors help give insights into the young geological history of mountain building in the tropical Americas (Fig. 1).

1) Oceanic lithosphere is being created at the Eastern Pacific Rise and Galapagos Spreading Center, resulting in two oceanic plates, the Cocos and Nazca, that are moving away from each other.
2) The North American and South American Plates are moving westward toward the Pacific Ocean, resulting in the subduction of the Cocos and Nazca Plates at the Middle American Trench (MAT) and the Peru-Ecuador-Colombian Trench (P-E-C T).
3) The Galapagos Hot Spot (GHS), a plume of magma derived from melting at deeper depths than melting at the Galapagos Spreading Center, has been erupting lavas on the Pacific Ocean floor for at least the last 20 Ma. Initially, the GHS was under the Nazca Plate and submarine lavas and small volcanoes have left a volcanic track of thickened crust as the Nazca moved over the GHP. Eventually the GSC moved over the GHS and began adding its lavas to the GSC lavas, creating thicker oceanic crust and two distinct ridges oriented parallel to the directions of plate motions relative to the GHP position: the ~NE-Trending Cocos Volcanic Ridge (CCVR) on the Cocos Plate and the east-trending Carnegie Volcanic Ridge (CVR) on the Nazca Plate (Fig. 3).

Because subduction of ridges create a partial obstruction to subduction when they reach a trench, these two volcanic ridges caused near coastal tectonic deformation landward of the middle American Trench and Peru-Ecuador-Colombia Trenches. These two zones of deformation had profound effects in mountain building in Costa Rica and Panama and in

TABLE 1. Selected orchid species diversity in the Tropics.

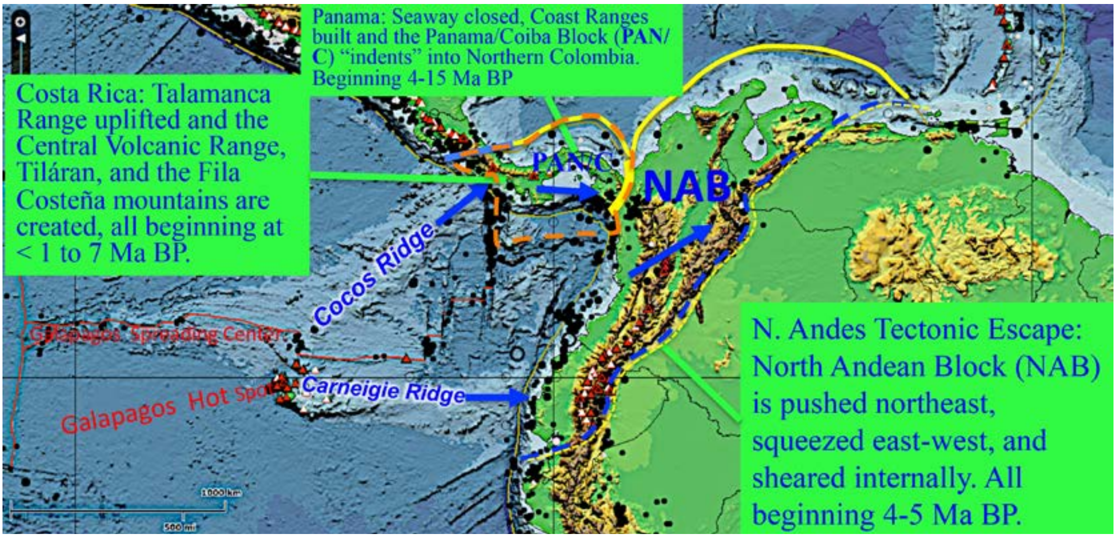
Country or Region	Estimated Number of Orchid Species	Land Area, 1000 km²	No. Species/1000 km²	Maximum Age of Elevation Start, Ma	Notes
New Guinea Island (Micro-Continent = West Papua + Papua New Guinea)	2759[1] 3200[2]	786 (~ 235 in the Central Cordillera)	3.5 (11.7) 4.1 (13.6)	~12	Severely undersampled; 98% endemic![1]
Ecuador	4056[3]	248 (128 minus the Amazonia lowland)	16.4 (31.7)	<15 acceleration at 4-6	~20% endemic[3]
Colombia	3960[4] 4270[5] 4354[6]	1197.4 (794 minus the Amazonia lowland)	3.3 (5.0) 3.6 (5.4) 3.6 (5.4)	4 and 15	Undersampled and underreported. ~37% endemic[6].
Panama	1360[7]	74.3	18.3	4 to 15 (Seaway closure)	Under-sampled especially east of Canal Zone. 21.7% endemic[7]
Costa Rica	1316[8] 1461[9] 1519[10] 1598[11]	51.1	25.7 28.6 29.7 31.3	0.5 to 7	Growth in species counts due to increased sampling effort and improved taxonomic methods. ~30% endemic[12]

References Cited in this Table

1. Schuiteman and de Vogel, (2001, 2002, 2005, 2006, 2008, 2010); 2. Papua New Guinea Orchid News [http://www.orchidspng.com/orfam.html accessed 9 March' 2016]; 3. Dodson, C. (1993-2004) ; 4. Sarmiento-Téllez (2006); 5. Betancour, Toro-González, and Valencia (2015); 6. Valencia and Betancour (2015); 7. Bogarín (2014); 8. Pupulin (2002); 9. Ossenbach, Pupulin, and Dressler (2007); 10. Bogarín (2011); 11. Bogarín et al. (2013); 12. Bogarín, D., personal communication on 10 March 2016.

the North Andes (Andean Ecuador-Colombia-Western Venezuela). Such collisions build tectonic mountains in several ways:

1) Collisions can create thrust faults and folds that shorten and thicken the crust and thereby raise elevations (Fig. 1). An example is the Fila Costeña and Talamanca Range of near-Pacific coastal Costa Rica. The birth of the Fila Costeña and the rejuvenation (young uplift) of the Talamanca Range are thought to be largely a consequence of the collision of the Cocos Ridge and rough seafloor of the GSC with the Pacific margin of Costa Rica. These events began sometime between late Neogene time and continued into the Quaternary (Fig. 2).
2) In some tectonic settings, microplates are created that move sideways of the collision direction, a process termed *tectonic escape* (Fig. 3 and 4). Part of the eastern motion of the Panama Block relative to South America may be attributable to eastward tectonic escape of the Panama Block (Microplate) (La Femina *et al.* 2009, La Femina 2011). A well-documented example is the tectonic escape of the North Andean Block (Microplate) (NAB) of Andean Ecuador and Colombia (Fig. 3) that is shown by the measured motions of this Block relative to the interior of the South American plate (Amazonia) based on GPS measurements (Fig. 4). Those motions may be resolved into internal strains in the NAB: an internal shear ~ parallel to the Colombian Trench and a shortening perpendicular to the Trench. These deformations are accomplished by strike-slip faulting (having horizontal slip parallel to the ~NE-trending vertical fault plane) and thrust faulting roughly perpendicular to the Colombian Trench. The NAB is thrust over the Caribbean Plate along its northern boundary (the North Colombian Thrust zone. The beginning of the collision of the Carnegie Ridge and the creation of the NAB has been dated from marine geology evidence of the age of the initial opening of the Guyaquil Canyon, a rift on the trailing edge of the



Panama: Seaway closed, Coast Ranges built and the Panama/Coiba Block (PAN/C) “indents” into Northern Colombia. Beginning 4-15 Ma BP

Costa Rica: Talamanca Range uplifted and the Central Volcanic Range, Tiláran, and the Fila Costeña mountains are created, all beginning at < 1 to 7 Ma BP.

N. Andes Tectonic Escape: North Andean Block (NAB) is pushed northeast, squeezed east-west, and sheared internally. All beginning 4-5 Ma BP.

Figure 3. Plate-tectonic causes of young mountain building in the Tropical Americas. Blue arrows indicate motions relative to the South American Plate (SAM). The two major drivers of these geological changes are: 1) Collision of the volcanic ridges produced by the Galapagos hot spot with Costa Rica and S. America and collision of the rough seafloor produced by the Galapagos Spreading Center and 2) Collision of the Panama/Coiba Block (PAN/C) and the North Andean Block (NAB) with South America, beginning in the time interval 4-15 My BP. These mountains have high elevations: Colombia and Ecuador have 20 peaks above 5000 m. Western Venezuela has 2 peaks near 5000 m. Costa Rica and Panama have 6 peaks above 3400 m. Same color symbols as in Fig. 1. Blue arrows represent motions of the PAN/C and NAB with the South American Plate.

NAB: 4 to 6 Ma BP (Collot *et al.* 2009, Nocquet *et al.* 2014). The combined squeezing and shearing motions in Andean Ecuador/Colombia/Western Venezuela (Nocquet *et al.* 2014) are evident in the pattern of earthquake distribution in the region (Figure 5), especially on the eastern margin of the NAB. Although the ancestral Andes certainly were built well before 4-6 Ma BP, most of the present-day high elevations of the northern Andes can be ascribed to the tectonic effects of the collision of the Carnegie Ridge. It is also worthwhile that many other independent observations, such as the dating of Andean uplift by fission-track methods and the onset and acceleration in sedimentation in the Amazon delta derived largely from the Andes show that such dates are in the same timeframe of tectonic uplifts discussed above (Hoorn *et al.* 2010).

3) Mountains can also be built by volcanic action at volcanic centers (red triangles in the maps of Fig. 1 and 3). Volcanic chains in subduction zones tend to be stratovolcanoes built by composites of lava flows and volcanic ash (technically tephra) and have the conical shapes of the famous Japanese straovolcano, Mount Fuji. There are 28 stratovolcanoes in the volcanic chains of Ecuador and Colombia with elevations above 3900 m and nine active stratovolcanoes in Costa Rica and western Panama with peak elevations between 1900 and 3500 m (Siebert *et al.* 2011). An important point is that arc volcanoes of this type grow in elevation and are, in turn, diminished by giant explosive eruptions and erosion (Kirby 2007, 2011). Moreover, their locations relative to their nearby trenches typically change over geologic time (Scholl & von Huene 1997). These changes therefore represent environmental changes associated with changes in elevation, rainfall, etc. in ways similar to tectonic mountains. And these changes can be extraordinarily fast. For example, the active Central Volcanic Range in Costa Rica with peak elevations as high as nearly 3500 m is less one Ma old. Arc volcanic ranges can appear and disappear depending on the nature of the incoming oceanic plate, including its deep shape after subduction. The Andes in South America

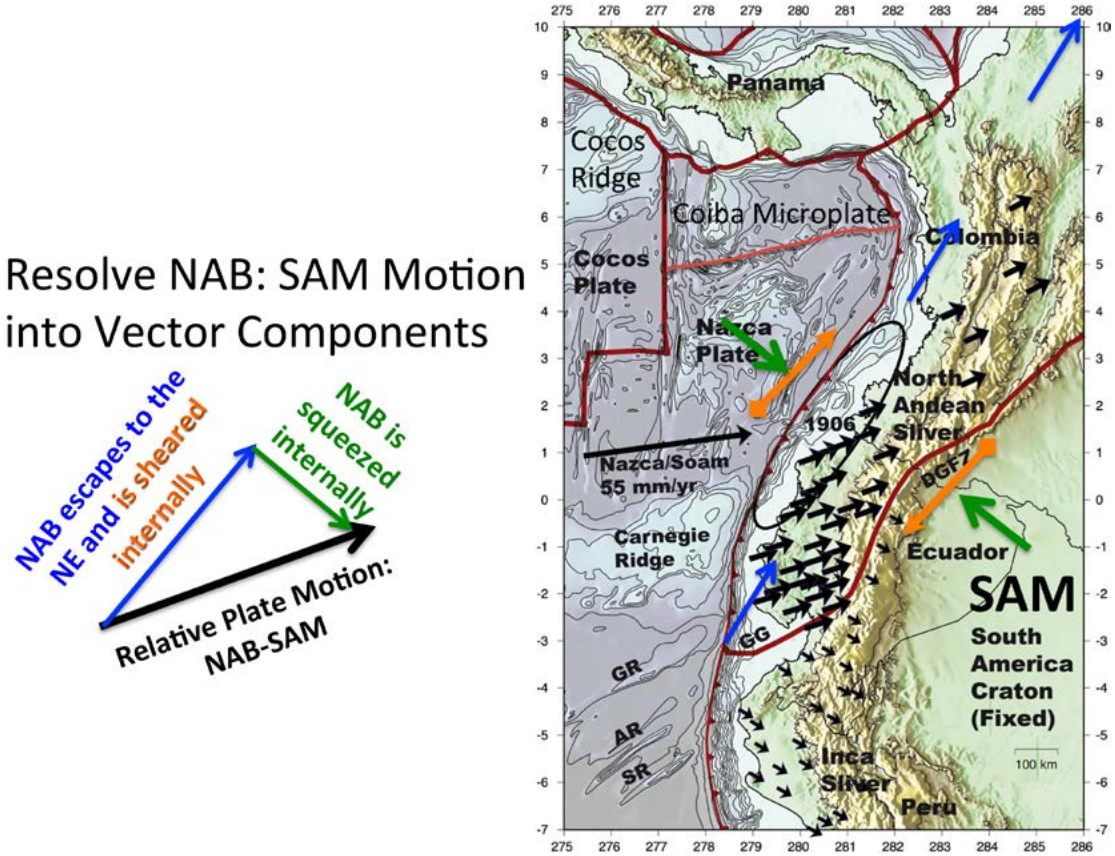


FIGURE 4. GPS measurements give insights into the most-recent speedup in the uplift of the northern Andes at 4 to 5 My BP when the Carnegie Ridge starts to collide with South America (Collot, 2009) and the North Andean Block (a microplate) begins its tectonic escape by moving north-northeast while being sheared and squeezed. See text. After Noquet *et al.* (2014, with permission).

show a rich history of such changes and as such should be considered part of the environmental drivers for speciation of orchids and other biota.

After this paper was submitted and reviewed, the author attended the Regional Assembly [of] Latin American and Caribbean Seismological Commission (LACSC) in San Jose, Costa Rica (20-22 June 2016) [www.lacsc2016.com]. While the general tectonic framework described this paper was confirmed by the presentations at this meeting, new observations from seismology, geology, and GPS measurements promise to provide additional insights into the detailed time evolution of mountain building in the neotropics.

A counterpart to this tectonic nexus in the tropical Americas exists in tropical Asia along the Island of New Guinea (West Papua, Indonesia + Papua New Guinea) and its environs (Fig. 6). There the Australia Plate is colliding with the Pacific Plate (here including the Carolyn Plate) in a broad Plate-Boundary Deformation Zone (PBDZ) prominently marked by a 2500-km long mountain chain (collectively called the Central Range) with 50 peaks having elevations above 3750 m (the highest in Australasia). Like the New World nexus just described, the deformation in this PBDZ represents a combination of squeezing and shearing deformation in a region of microplates the relative motions of which are producing some of the highest uplift rates in the world; just 12 Ma BP, the island was largely under water! (Baldwin *et al.* 2012). Like Colombia, the orchids of New Guinea Island are severely undersampled, but show similar promise of much larger species counts and

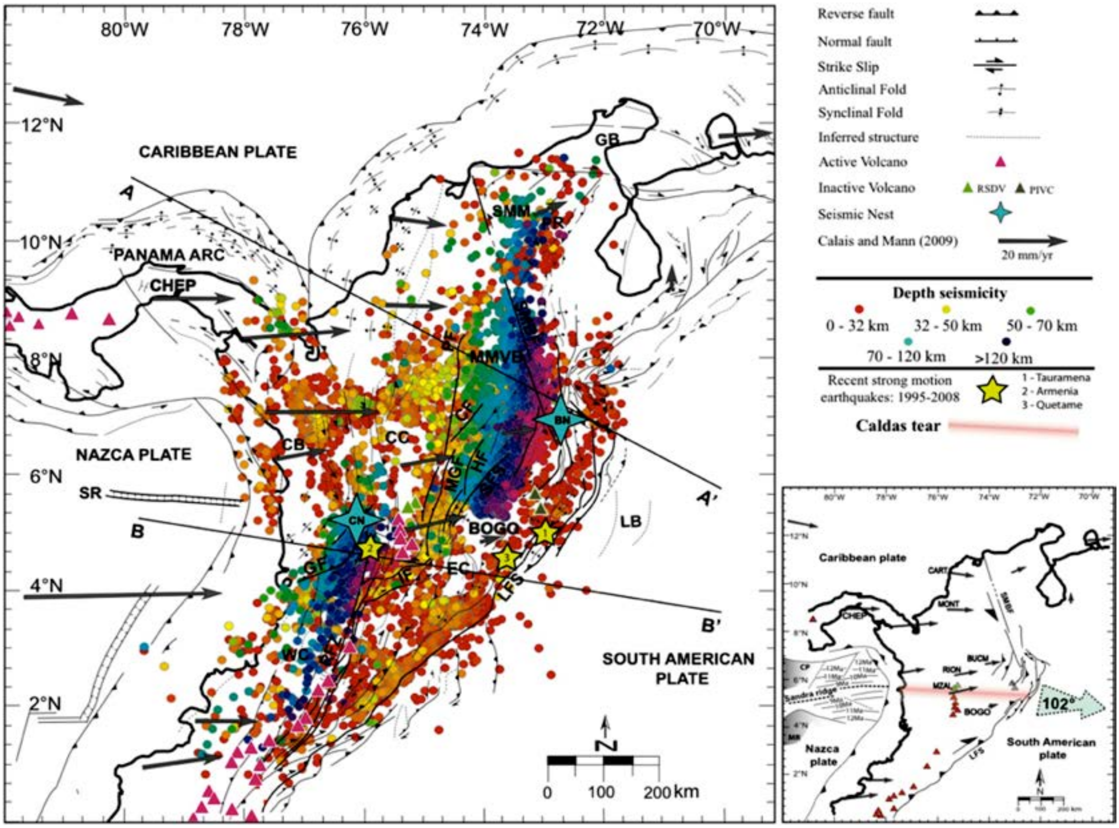


Figure 5. (a) Seismicity of Colombia and Western Venezuela. Figure from Vargas and Mann (2013, with permission). Shallow earthquakes (red and yellow dots) mark the thrust- and strike-slip motions between the Colombian microblocks caused by the internal deformation of the North Andean Block (data from the Colombian National Seismic Network). These belts of earthquakes mark the eastern, central, and western cordilleras and demonstrstrate that these mountain ranges are presently tectonically active. The intermediate-depth earthquakes (green, blue, and black dots) occur in a zone dipping to the east and is offset by the Caldas tear in the Nazca Plate that aligns with the offshore Sandra Ridge. (b) GPS measurements of crustal motion relative to the South American Plate (gray arrows with labels) consistent with the internal deformation of the North Andean Block (Fig. 4).

area density, especially in its cordillera (Table 1). The timing of speciation events for these plants and animals described in the next section show parallels with timing of geologic events in the tropics that I have reviewed above.

Timing of speciation in orchids and their pollinators. In October 2015, just before the V Scientific Conference on Andean Orchids in Cali, Colombia, I became aware of an important paper on molecular phylogenetic study of orchids by Tom Givnish and 15 others (Givnish *et al.* 2015), who sampled selected species from all of the orchid subfamilies. Although I do not have the technical background to judge the paper's methodology and also the number of species was limited, it was a multi-institutional collaboration of respected orchid scientists in the molecular phylogenetics, some of them pioneers in the field. Finally it was peer reviewed and published in a prestigious scientific journal. Although I have not parsed out the species endemic to the high elevations of the cordilleras and high intermontaine valleys in the neotropical nexus discussed above, the preponderance of neotropical species sampled by the authors occur there.

Their results are shown in Fig. 7, adapted from the Epidendroideae section of their Fig. 3. The vertical widths of the red horizontal lines are proportional to the rates of diversification (speciation rates minus

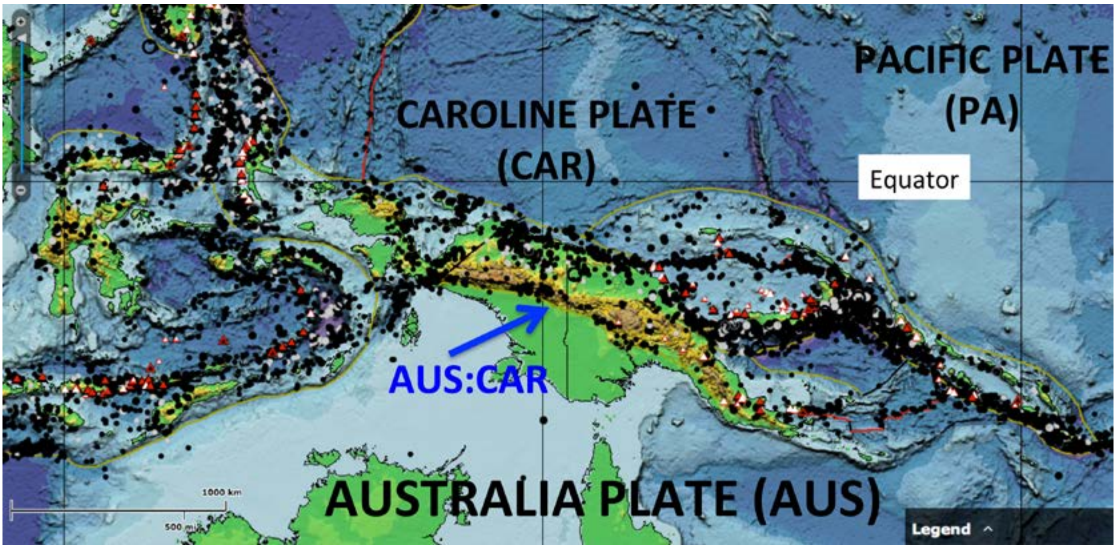


Figure 6. New Guinea Island: A tectonic counterpart in Asia of the NAB and Panama Block. The island is made up of West Papua (Irian Jayra, Indonesia) and Papua New Guinea). Along its spine is a 2000-km-long mountain chain that has the highest ongoing uplift rates in the world. This region was underwater as recently as 12 My ago. The range has 50 peaks above 3750 m, the highest at 4884 m. The Island also has among the most diverse orchid flora in the world. Same symbols as Figs. 1 and 2. Constructed from the online *This Dynamic Planet* online tools (http://nhb-arcims.si.edu/ThisDynamicPlanet/). Same symbols as Fig. 1 and 3.

extinction rates). I also indicate graphically which subfamilies are New World and which are Old World. Immediately evident among the Epidendroideae are the accelerations of diversification beginning at about 15 Ma BP and additional accelerations at 4-7 Ma BP.

These authors consider a number of factors that contribute to these jumps in diversification based on where the sampled species came from and their spatial distribution, what fauna pollinate them, their habitats, whether they are CAM, etc. They specifically identify the following factors as significant in descending order of significance:

A. *Geographical factors*: *a*) Distribution in tropical cordilleras, notably in the tropical Americas and New Guinea. *b*) tropical distribution.
B: *Biological factors*: i) CAM metabolism; ii) Epiphytism; iii) Pollinia pollination transport; iv) Other factors, such as deceit pollination, pollination by Lepidoptera and Euglossine bees.

Epiphytism itself is possibly linked to tropical cordillera through the orographic effects of mountain chains in increasing rainfall and producing high humidity and their effects on forest growth and vertical zonation of microclimates.

The chronology of diversification rates in preliminary results described in the pioneering paper by Givnish *et al.* (2015) are very exciting to this author because the timing of accelerations correspond to the geologic timing of mountain building events. A productive area of research is in the interactions of factors due to place, such as tropical climate and mountains and those due to biological changes. It is interesting that parallel studies of the chronologies of diversification of known pollinators known to pollinate orchids, such as euglossine bees (Ramírez *et al.* 2010) and some hummingbirds (McGuire *et al.* 2014). It is noteworthy that many of the branch points in the diversification of these orchid pollinators in their centers of diversity of these fauna are bracketed within the mid-Miocene to Quaternary (15 Ma to 0 Ma), a geological time frame for tectonic and volcanic events in the nexus of neotropical mountain building. This area of research is likely a productive direction, but further developments and refinements are beyond the scope of this paper.

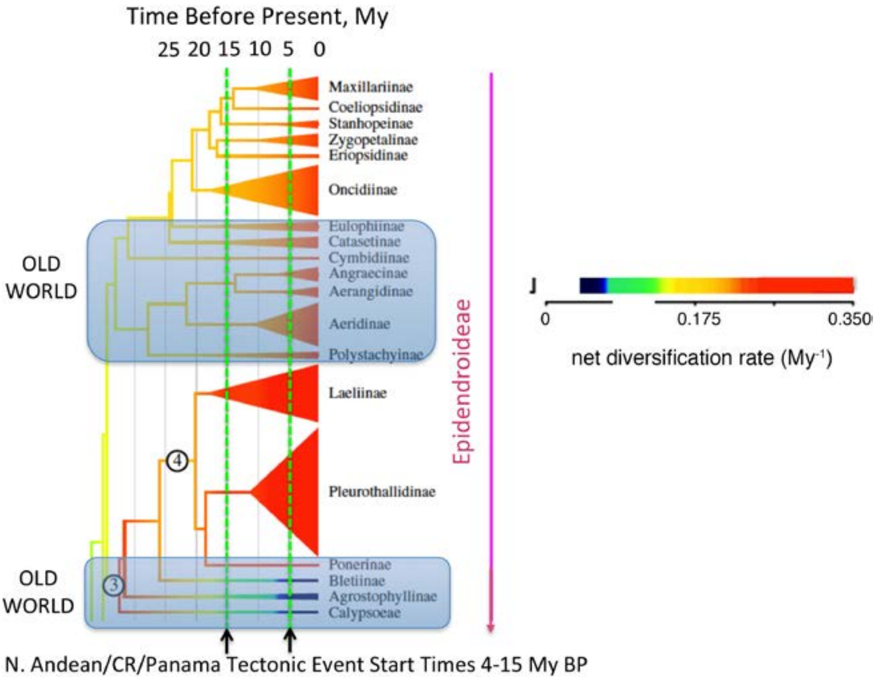


Figure 7. Neotropical orchid diversification rates for Epidendroideae over time based on phylogenetic data (adapted from Givnish et al., 2015, with permission). Subfamilies from the Old World are marked with enclosing boxes, otherwise the subfamilies are neotropical.

Possible insights into long-distance orchid dispersal between Central America and South America and the urgent need for additional information on sampling, taxonomy, and information flow about orchid species in eastern Panama and Andean Colombia. In the IV Scientific Conference on Andean Orchids in Guyaquil Ecuador, Kirby *et al*. (2012) described an attempt to investigate the biogeography of orchid species in the genus *Camaridium* (Orchidaceae: Maxillariinae). One of the motivations was to investigate the gene flow between SE Central America where most of the estimated 80 species in this genus are found and the northern Andes (Whitten *et al*. 2007, Blanco *et al.* 2007). Even though we authors collected nearly 2400 geographic distribution sites based on data from herbarium sheets, national and regional flora, and national and regional monographs, the results were only partially successful because of only a few dozen distribution sites in eastern Panama and Andean Colombia for only a handful of *Camaridium* species. This created an E-W gap of nearly 900 km between western Panama and Venezuela and N-S gap of more than 1000 km between western Panama and Ecuador for most of the known *Camaridium* species. Fortunately, the situation is rapidly changing due to purposeful collecting and taxonomy and to compilation of diverse information sources. Diego Bogarín and his Lankester Garden, and Panamanian and other coauthors recently published an impressive national checklist of orchids in Panama compiled from herbarium vouchers and published taxonomic treatments (Bogarín *et al.*, 2014). Although sampling in eastern Panama is still relatively sparse compared to western Panama, Bogarín also described developing efforts to widen collaborative collections and identifications in eastern Panama that promise to help fill in this gap. In Colombia, the situation is also changing with the monumental national publication written by Betancourt, Sarmineto, Toro-Gonzâlez, and Valencia (First edition, April 2015) under the auspices of Colombian Ministry of the Interior and the Institute of Natural Sciences of the National University of Colombia in Bogotá. This publication used many of the same types of information sources as the earlier Bogarín *et al.* (2014) publication. Janet

Valencia and Julio Betancur (Abstracts in V Scientific Conference on Andean Orchids in Cali, 2015) did an initial analysis of the patterns of diversity and biogeography from the results of this new compilation and promised that important work will continue and be updated with subsequent editions. Some of the sources of information in the 2015 publication and presentation came from the publication of regional monographs and checklists over the last decade or so, including Misas (2005), the Sociedad Colombiana de Orquidiología (2015), Ortíz Valdivieso and Uribe Vélez (2014), and many others published by the regional orchid societies in Colombia.

One of the sections of my 2015 presentation (Abstracts V Scientific Conference in Cali) included a discussion of the likely mechanisms of long-distance dispersal of orchids in the tropical Americas. These include:

1) Animal migration by birds and mammals;
2) Extreme winds in cyclones;
3) Downstream transport of log rafts during seasonal and cyclonic floods;
4) Earthquake-induced breaching of natural dams caused by huge landslides (e.g., 1987 in Ecuador);
5) Log-raft transport by shallow ocean currents.

All of these mechanisms are to varying extents seasonal and directional (largely from east to west). Moreover it should also be noted that the control of intermontaine valleys by the tectonic and volcanic processes described earlier also control the directions of movement of log rafts during floods, largely to the north-northeast in Colombia. When geographical sampling and taxonomic studies discussed earlier in this section are more advanced, it should be possible to use the seasonal and directional natures of these dispersal processes for hypothesis testing.

How many orchid species are there in Colombia? Since it is well established that mountains are crucibles for orchid diversity, an approach to estimate how many orchid species exist (or existed prior to European contact) in Colombia might be had by comparing the comparative lengths of cordilleras in Colombia with the length of the principal cordillera in Ecuador that has an estimated number of about 4000 known species. For Ecuador, the combined length of cordilleras is about 600 km, whereas in Colombia, the multiple cordilleras total about 1900 km. So this crude estimate suggest that there are (1900/600 x 4000) = about 12,700 orchid species in Colombia, But not all cordilleras are equal in average width, elevation, rainfall, yearly air temperatures, and latitudinal variations in westward winds from the Amazon and the Caribbean. For example, the main mountain chain in Ecuador is wider than all but one segment of the Colombian mountain chains. Allowing for these differences, perhaps this crude estimate of 10,000 orchid species in Colombia is a starting point on what to expect eventually should orchid surveys and taxonomic identifications trend in the ways that I hope continue.

Acknowledgements. I am indebted to my colleagues in orchid science for their knowledge and enthusiasm and encouragement of my research that have been the things that guide my continuing education in this field. I especially acknowledge my colleagues and friends at Lankester Gardens of the University of Costa Rica and their research associates: Franco Pupulin, Adam Karremans, Melissa Díaz, Diego Bogarín, Mario Blanco, and architect Carlos Ossenbach. I also thank current and former Orchid Botanists in Charge at Bosque de Paz Biological Reserve in Costa Rica: Melania Muñoz-Garcia, Adam Karremans, Melissa Díaz, and Orelia Belfort-Oconitrillo. It is my pleasure to call out the important contributions by James Ackerman to orchid science and orchid biogeography and his help in identifying Greater Antilles collection sites for species in the genus *Camaridium*. I also thank the Organizing Committee chaired by Nicola Sian Flanagan for inviting me to the Cali Conference and Alec Pridgeon and the other Organizing Committees for the Quito and Guyaquil Conferences for earlier invitations for invitations to speak at those Conferences. Finally, I have already acknowledged in the Prologue section the valuable insights of Calaway Dodson into some of the roles of geology in orchid diversity and biogeography.

Literature Cited

Ackerman, J. D. (2012). Abstract in the IV Andean Orchid Conservation Conference. Guayaquil, Ecuador.

Ackerman, J. D. (2014). Rapid transformation of orchid floras. *Lankesteriana,* 13(3), 157-164. doi: 10.15517/lank.v13i3.14349

Baldwin, S. L., Fitzgerald, P. G. & Webb, L. E. (2012). Tectonics of the New Guinea Region. *Annual Review of Earth and Planetary Sciences,* 40, 495-520. doi: 10.1146/annurev-earth-040809-152540

Betancur, J., Sarmiento, H., Toro-González, L. & Valencia, J. (2015). *Plan para el estudio y la conservación de las* *orquídeas en Colombia*. Bogotá: Universidad Nacional de Colombia.

Blanco, M. A., Carnevali, G., Whitten, W. M., Singer, R. B., Koehler, S., Williams, N. H., Ojeda, I., Neubig, K. M. & Endara, L. (2007). Generic realignments in Maxillariinae (Orchidceae). *Lankesteriana,* 7(3), 515-537. doi: 10.15517/lank.v0i0.7935

Bogarín, D. (2011). How many orchid species in Costa Rica: A review of the latest discoveries. *Lankesteriana,* 11(3), 185-205. doi: 10.15517/lank.v11i3.18274

Bogarín, D., Pupulin, F., Arrocha, C. & Warner, G. (2013). Orchids without borders: studying the hotspot of Costa Rica and Panama. *Lankesteriana,* 13(1-2), 13-26. doi: 10.15517/lank.v0i0.11529

Bogarín, D., Serracín, Z., Samudio, Z., Rincón, R. & Pupulin, F. (2014). An updated checklist of the Orchidaceae of Panama, *Lankesteriana,* 14(3), 135- 364. doi: 10.15517/lank.v14i3.17958

Collot, J.-Y., Michaud, F., Alvarado, A., Marcaillou, B., Sosson, M., Ratzov, G., Migeon, S., Calahorrano, A. & Pazmino, A. (2009). Visión general de la morfología submarina del marge convergente de Ecuador – Sur de Colombia: implicaciones sobre la transferencia de masa y la edad del a subducción de la Cordillera de Carnegie. En: Collot, J.-Y., Sallares, V. & Pazmino, A. (Eds.), *Geología y geofísica marina y terrestre del Ecuador: desde la costa continental hasta las Islas Galápagos*. Guayaquil: INOCAR.

Dodson, C. & Escobar, R. (1993). *Native Ecuadorian Orchids. Vol 1: Aa-Dracula*. Brasil: Colina Livraria Editora.

Dodson, C. (2001). *Native Ecuadorian Orchids. Vol 2: Dresslerella-Lepanthes*. Sarasota: Dodson Publishing.

Dodson, C. (2002). *Native Ecuadorian Orchids. Vol 3: Lepanthopsis-Oliveriana*. Sarasota: Dodson Publishing.

Dodson, C. (2003). *Native Ecuadorian Orchids. Vol 4: Oncidium-Restrepiopsia*. Sarasota: Dodson Publishing.

Dodson, C. (2004). *Native Ecuadorian Orchids. Vol 5: Rodriguezia-Zygosepalum*. Sarasota: Dodson Publishing.

Dodson, C. H. (2003). Why are there so many orchid species? *Lankesteriana,* 3(2), 99-103. doi: 10.15517/lank.v3i2.23028

Givnish, T. J., Spalink, D., Ames, M., Lyon, S. P., Hunter, S. J., Zuluaga, A., Iles, W. J. D., Clements, M. A., Arroyo, M. T. K., Leebens-Mack, J., Endara, L., Kriebel, R. Neubig, K. M., Whitten, M. W., Williams, N. H. & Cameron, K. M. (2015). Orchid phylogenomics and multiple drivers of their extraordinary diversification. *Proceedings of the Royal Society B,* 282, 1-10. doi: 10.1098/rspb.2015.1553

Hoorn, C., Wesselingh, F. P., ter Steege, H., Bermudez, M. A., Mora, A., Sevink, J., Sanmartín, I., Sanchez-Meseguer, A., Anderson, C. L., Figuereido, J. P., Jaramillo, C., Riff, D., Negri, F. R., Hooghiemstra, H., Lundberg, J., Stadler, T., Särkinen, T. & Antonelli, A. (2010). Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science,* 330, 927-931.

Kirby, S. H. (2007). Geological processes and orchid biogeography with applications to southeast Central America. *Lankesteriana,* 7(1-2), 53-55. doi: 10.15517/lank.v7i1-2.18436

Kirby, S. H. (2011). Active mountain-building and the distribution of 'core" Maxillariinae species in tropical México and Central America. *Lankesteriana,* 11, 275-291. doi: 10.15517/lank.v11i3.18283

Kirby, S. H., Blanco, M., Muñoz-Garcia, M., & Villalobos, F. (2012). A preliminary biogeographic study of the Neotropical genus *Camaridium* (Orchidaceae: Maxillariinae). Abstract IV Scientific Conference on Andean Orchids. Guyaquil, Ecuador.

LaFemina, P. (2011). ICTP Workshop Report: Seismic Sources in Central America. Heredia, Costa Rica. Retrieved from http://indico.ictp.it/event/a10192/material/0/14.pdf

LaFemina, P., Dixon, T. H., Govers, R., Norabuena, E., Turner, H., Saballos, A., Mattioli, G., Protti, M. & Strauch, W. (2009). Fore-arc motion and Cocos Ridge collision in Central America. *Geochemistry, Geophysics, Geosystems,* 10, 1-21.doi: 10.1029/2008GC002181.

Mann, P. (1995). Geologic and tectonic development of the Caribbean plate boundary in Central and South America. *Geological Society of America Special Paper,* 295, 1-349.

Mann, P. & Corrigan, J. (1990). Model for late Neogene deformation in Panama. *Geology,* 18, 558-562.

Mann, P., Rogers, R. D. & Gahagan, L. (2006). Overview of plate tectonic history and its unresolved tectonic problems. In: J. Bundschuh & G. E. Alvarado (Eds.), *Central America: geology, resources and hazards, vol. 1*. (pp. 201-232). London: Taylor and Francis.

McGuire, J. A., Witt, C. C, Remsen, J. V, Jr., Cori, A., Rabosky, D. L., Altshuler, D. L. & Dudley, R. (2014). Molecular phylogenetics and the diversification of hummingbirds. *Current Biology,* 24, 910-916.

Misas-Urreta, G. (2006). *Orquídeas de la Serranía del Baudó, Chocó, Colombia*. Bogotá: Corporación Capitalina de Orquideología.

Nocquet, J-M., Villegas-Lanza, J. C., Chlieh, M., Mothes, P. A., Rolandone, F., Jarrin, P., Cisneros, D., Alvarado, A., Audin, L., Bondoux, F., Martin, X., Font, Y., Régnier, M., Vallée, M., Tran, T., Beauval, C., Maguiña Mendoza, J. M., Martinez, W., Tavera, H. & Yepes, H. (2014). Motion of continental slivers and creeping subduction in the northern Andes. *Nature Geoscience,* 7, 287-291. doi: 10.1038/ngeo2099

Ortiz-Valdivieso, P. & Uribe Vélez, C. (2014). Orquídeas, tesoro de Colombia / Orchids – a Colombian treasure. Bogotá: Da Vinci Publicidad y Medios. 398 p. ISBN: 978-958-46-5329-1.

Ossenbach, C., Pupulin, F. & Dressler, R. L. (2007). *Orquídeas del istmo Centroamericano: catálogo y estado de conservación*. Sabanilla, Costa Rica: Editorial 25 de Mayo.

Pupulin, F. (2002). Catálogo revisado y anotado de las Orchidacaceae de Costa Rica. *Lankesteriana,* 2(2), 1-88. doi: 10.15517/lank.v2i2.23095

Ramírez, S. R., Roubik, D. W., Skov, C. & Pierce, N. E. (2010). Phylogeny, diversification patterns and historical biogeography of euglossine orchid bees (Hymenoptera: Apidae). *Biological Journal of the Linnean Society,* 100, 552–572.

Sak, P. B., Fisher, D. M., Gardner, T. W., Marshall, J. S. & LaFemina, P. C. (2009). Rough crust subduction, forearc kinematics, and Quaternary uplift rates, Costa Rica segment of the Middle American Trench. *Geological Society of America Bulletin,* 121, 992-1012.

Schuiteman, A. & de Vogel, E.F. (2001). Orchids of New Guinea I. National Herbarium of the Netherlands, University of Leiden Branch. Retrieved from http://www.nhn.leidenuniv.nl

Schuiteman, A. & de Vogel, E.F. (2002). Orchids of New Guinea II. National Herbarium of the Netherlands, University of Leiden Branch. Retrieved from http://www.nhn.leidenuniv.nl

Schuiteman, A. & de Vogel, E.F. (2005). Orchids of New Guinea III. National Herbarium of the Netherlands, University of Leiden Branch. Retrieved from http://www.nhn.leidenuniv.nl

Schuiteman, A. & de Vogel, E.F. (2006). Orchids of New Guinea IV. National Herbarium of the Netherlands, University of Leiden Branch. Retrieved from http://www.nhn.leidenuniv.nl

Schuiteman, A. & de Vogel, E.F. (2008). Orchids of New Guinea V. National Herbarium of the Netherlands, University of Leiden Branch. Retrieved from http://www.nhn.leidenuniv.nl

Schuiteman, A. & de Vogel, E.F. (2010). Orchids of New Guinea VI. National Herbarium of the Netherlands, University of Leiden Branch. Retrieved from http://www.nhn.leidenuniv.nl

Siebert, L., Simkin, T. & Kimberly, P. (2011). *Volcanoes of the World*, Third Edition. California: University of California Press. 568 p. [ISBN: 9780520268777].

Simkin, T., Tilling, R., Vogt, P., Kirby, S. H., Kimberly, P. & Stewart, D. B. (2006). This dynamic planet – world map of volcanoes, earthquakes, impact craters, and plate tectonics, U. S. Geological Survey Geologic Investigations Map I-2800, Washington, D.C., USA. Map and Poster. Online interactive version http://mineralsciences.si.edu/tdpmap/

Sociedad Colombiana de Orquideología (Ed.). (2014). *Orquídeas del Valle de Aburrá, Medellín. Colombia*. Medellín: SCO.

Valencia, J. & Betancur, J. (2015). Exploring the diversity patterns of orchids in Colombia. Abstract V Scientific Conference on Andean Orchids. Guyaquil, Ecuador.

Vargas, C.A. & Mann, P. (2013). Tearing and breaking off of subducted slabs as the result of collision of the Panama Arc-Indenter with Northwestern South America. *Bulletin of the Seismological Society of America*, 103, 2025-2046. doi: 10.1785/0120120328

von Huene, R. & Scholl, D.W. (1991). Observations at convergent margins concerning sediment subduction, sediment erosion, and the growth of continental crust. *Reviews of Geophysics,* 29, 279–316. doi: 10.1029/91RG00969

Whitten, W. M., Blanco, M. A., Williams, N. H., Koehler, S., Carnevali, G., Singer, R. B., Endara, L. & Neubig, K. M. (2007). Molecular phylogenetics of *Maxillaria* and related genera (Orchidaceae: Cymbidieae) based on combined molecular data sets. *American Journal of Botany*, 94(11), 1860-1889. doi: 10.3732/ajb.94.11.1860

LANKESTERIANA 16(2): 255–267. 2016. **doi**: http://dx.doi.org/10.15517/lank.v16i2.26010

RECENT ADVANCES ON EVOLUTION OF POLLINATION SYSTEMS AND REPRODUCTIVE BIOLOGY OF VANILLOIDEAE (ORCHIDACEAE)

Emerson R. Pansarin

Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo,
Av. Bandeirantes 3900, 14040-901, Ribeirão Preto, SP, Brazil • epansarin@ffclrp.usp.br

Abstract. Vanilloideae as currently circumscribed comprises nine genera and two tribes: Vanilleae and Pogonieae. The pantropical genus *Vanilla* has been frequently assumed to be natural on the basis of its climbing habit and lateral inflorescences. However, the inclusion of the rare *Dictyophyllaria dietschiana* in phylogenetic analyses makes the genus *Vanilla* paraphyletic. Within Pogonieae, phylogenetic analyses show that inclusion of *Pogoniopsis* turns the tribe paraphyletic. All analyses reveal that *Pogoniopsis* is closely related to members of Epidendroideae. Members of Pogonieae are pollinated by several groups of solitary and social bees, two pollination systems being recognized: reward-producing and deceptive. Molecular phylogeny suggests that the common ancestor to Pogonieae gave rise to two evolutionary lineages: one tropical with a condition of reward production; and one predominantly temperate-invading line with deceptive flowers. Reward-producing flowers characterize South and Central American clade (= *Cleistes*), while deceptive pollination is prominent in the clade including North American-Asiatic taxa plus Amazonian *Duckeella*. Species of "orchid bees" have been recorded as pollinators of the genus *Vanilla* (*V. planifolia* group and *V. pompona* group) in the Neotropics. In species of the *V. pompona* group, these bees are attracted by the fragrance of the flowers. Hummingbirds have been reported to pollinate some species of *Vanilla*. *Vanilla insignis*, *V. odorata* and *V. planifolia* are known to be pollinated through generalized food deception. Some species of *Vanilla* yield fruits through spontaneous self-pollination. This form of autogamy has been reported for *V. griffithii*, *V. palmarum*, *V. planifolia*, *V. savannarum* and *V. bicolor*. In Brazil, data on the pollination biology of *Vanilla* are scarce, but conclusive data are available for *V. edwallii,* which is pollinated by *Epicharis* (Apidae: Centridini). This species is rewardless, but male *Epicharis* are attracted to its flowers by their fragrance. Additionally, the Brazilian *V. dubia* and *E. sclerophyllum* are pollinated by bees. The mentum region of *V. dubia* and *V. edwallii* is dry, whereas that of *E. sclerophyllum* presents a small quantity of nectar. Flowers of *E. sclerophyllum* are scentless, while those of *V. dubia* are odoriferous. *Vanilla dubia* and *V. edwallii* are self-compatible and need a pollinator to yield fruits. In contrast, *Epistephium sclerophyllum* sets fruits through spontaneous self-pollination, but biotic pollination also occurs. Both species are primarily adapted to pollination by euglossine bees. Pollination by Euglossini seems to have evolved at least twice along the evolution of Vanilleae. Furthermore, shifts between rewarding and rewardless flowers and between autogamous and allogamous species have been reported among vanillas.

Key words: Flower reward, food deception, Pogonieae, pollination biology, reproduction, Vanilleae

Phylogenetic relationships within Vanilloideae. Since the first comprehensive study published by Darwin (1892), the pollination systems displayed by orchid flowers have inspired biologists over the centuries. Nowadays, several research groups have focused on understanding pollination biology and evolution of pollination systems of orchids based on the combination of phylogenic hypothesis with morpho-anatomical studies and several kinds of ecological data (see Pansarin *et al.* 2012). In some orchid groups, however, these data are very difficult to be obtained since many pecies are not cultivable and investigations need to be made exclusively in the field. This is the case for Vanilloideae, a subfamily where data on the natural history of several genera, such as *Cleistes* for instance, must be collected exclusively in the natural environment.

Received 3 May 2016; accepted for publication 3 July 2016. First published online: 30 August 2016.

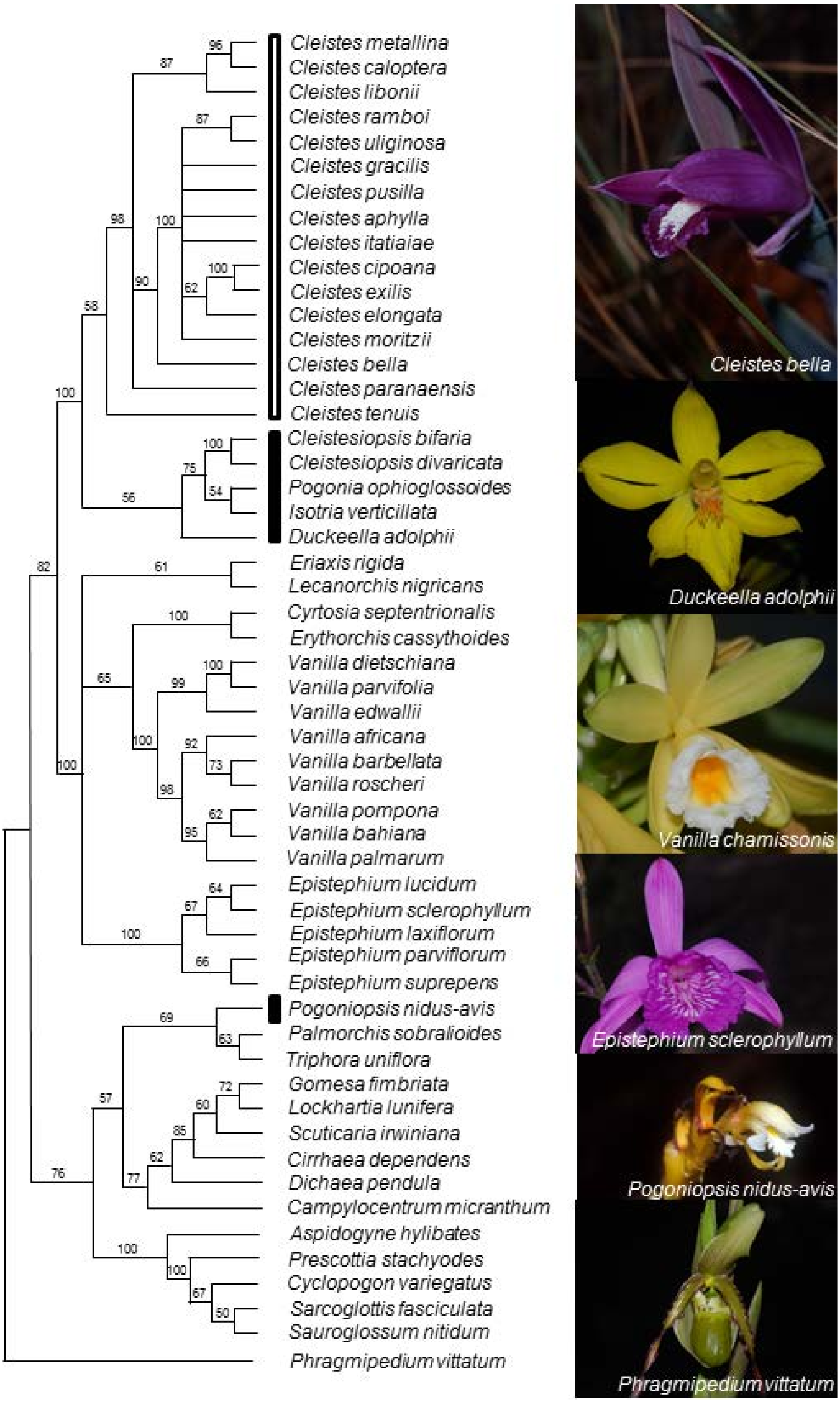


FIGURE 1. Maximum parsimony analyses based on combined 5.8S, 18S and 26S (nrDNA) regions of Pogonieae (Orchidaceae, Vanilloideae). Bootstrap values >50 (MP) are given above branches. Vertical bars refer to tribe Pogonieae. Empty bars = nectar-producing Pogonieae (i.e., genus *Cleistes*). Full bars = rewardless Pogonieae. Note that Vanilloideae and Pogonieae, as currently circumscribed, are paraphyletic. Adapted from Pansarin *et al.* (2012).

Figure 2. *Vanilla dietschiana*. A. Habit. Note the stem with monopodial (pseudosympodial) growth; B. Flower. Note the green sepals and petals and the white tubular labellum with longitudinal keels on the median portion.

Vanilloideae has been considered as a monophyletic subfamily (Cameron, 2003, 2009). However, the inclusion of *Pogoniopsis* in the analysis turns Vanilloideae paraphyletic (Fig. 1; adapted from Pansarin *et al.* 2012). Vanilloideae is currently divided into the tribes Vanilleae and Pogonieae (Cameron 2003, 2009; Pansarin *et al*. 2012). Vanilleae is subcosmopolitan in distribution and comprises nine genera, namely *Clematepistephium, Cyrtosia, Epistephium*, *Eriaxis*, *Erythrorchis, Galeola*, *Lecanorchis, Pseudovanilla* and *Vanilla*. The genus *Vanilla* is pantropical and has been frequently assumed to be natural on the basis of its climbing habit and lateral inflorescences (Cameron & Molina 2006). However, the inclusion of the rare *Dictyophyllaria dietschiana* in the phylogenetic analyses makes the genus *Vanilla* paraphyletic (Pansarin 2010a, 2010b; Pansarin *et al*. 2012). Thus, *Dictyophyllaria* has been restored to *Vanilla* (Pansarin 2010a, 2010b; Pansarin *et al.* 2012). *Vanilla dietschiana* (Figs. 2A-B) is closely related to *V. edwallii* and *V. parvifolia* by floral and vegetative characters. *Vanilla dietschiana* is terrestrial, with monopodial (pseudosympodial) non-climbing habit. Its reduced leaves are pale green, reticulate-veined and membranous, such as in *V. edwallii* and *V. parvifolia* (Pansarin 2010b).

The North American-Asiatic Pogonieae includes five genera: *Cleistes*, *Cleistesiopsis, Duckeella, Isotria* and *Pogonia* (Pansarin *et al.* 2008, 2012). The genus *Duckeella,* which is endemic to Amazonia, frequently emerges as sister to the rest of Pogonieae (Pansarin *et al.,* 2008, Pansarin *et al.* 2012). The genera *Isotria, Pogonia,* and *Cleistesiopsis* (Pansarin & Barros, 2008) form a temperate group, sister to the tropical *Cleistes*, a robustly supported South-Central American clade (*e.g.,* Pansarin *et al.* 2008, Pansarin *et al.* 2012).

Pogoniopsis, a myco-heterotrophic genus endemic to Brazilian forests, has been included in the tribe Pogonieae (Cameron, 2003, 2009), which has been assumed as monophyletic (Cameron & Chase 1999; Cameron *et al.* 1999). However, a phylogenetic analyses show that inclusion of *Pogoniopsis* turns the tribe paraphyletic (Fig. 1; Pansarin *et al.* 2012). All analyses reveal that *Pogoniopsis* is closely related to members of Epidendroideae (Fig. 1; Pansarin *et al.* 2012). *Pogoniopsis* have a sympodial habit with reduced leaves. The inflorescence is a terminal raceme (Fig. 3A). The flowers are whitish and the

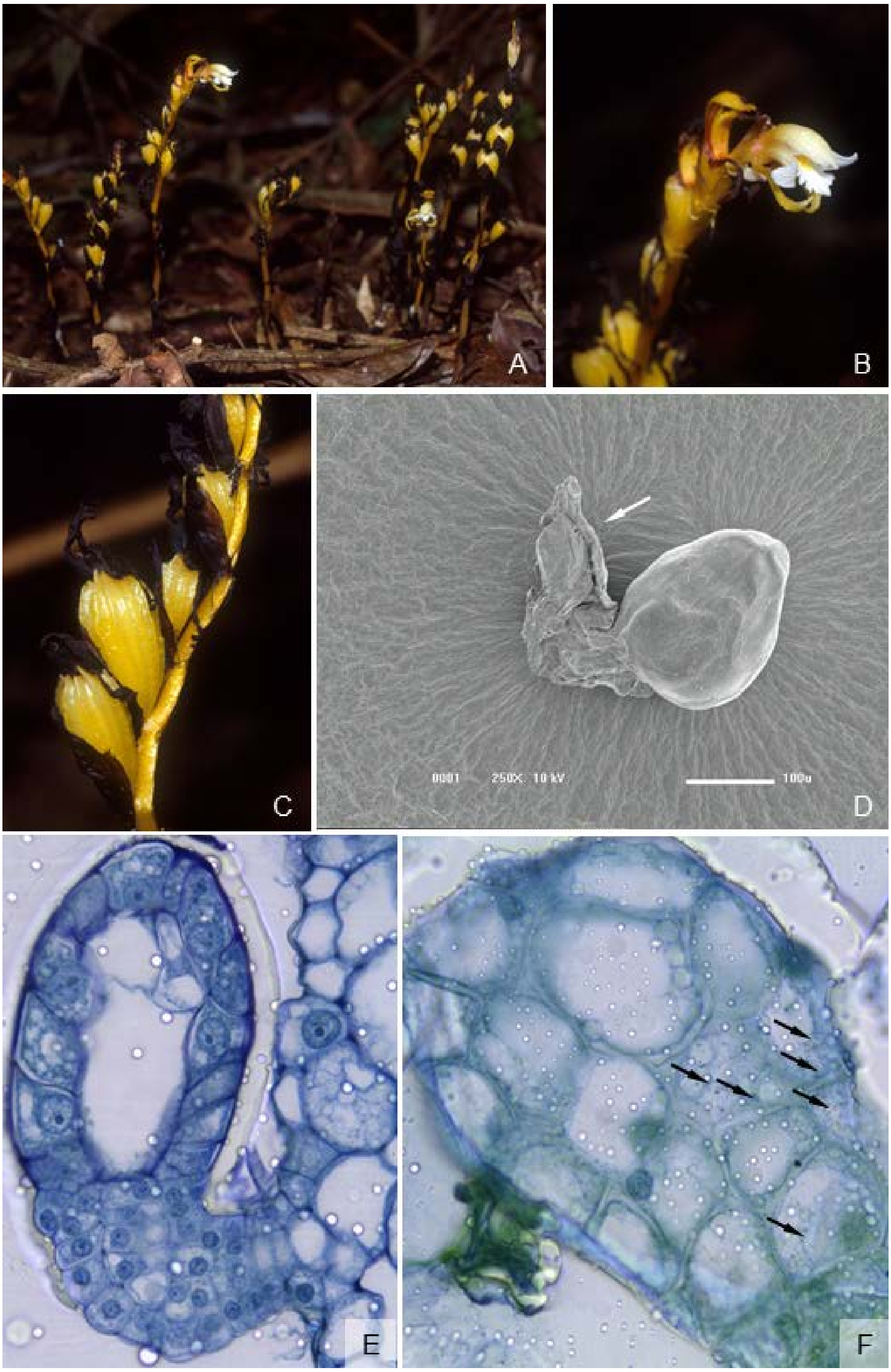


FIGURE 3. *Pogoniopsis nidus-avis*. A. Habit; B. Flower; C. Fruits (note the persistent perianth parts); D. Scanning Electron Microscopy (SEM) image of a sclerified seed. Note the remnant of the funiculus (arrow); E. Longitudinal cut of a seed stained with toluidine blue. Note the funiculus and the placenta; F. Longitudinal section of an ovule stained with toluidine blue showing the cells with large nuclei and densely stained cytoplasm. Note the granules, which are related to embryo nutrition (arrows).

perianth is persistent (Figs. 3A-B). The labellum is 3-lobed and presents clusters of yellow hairs on its mid portion. The lateral lobes are falcate and entire, while the apical lobe is spatulate and fringed. The pollen of *Pogoniopsis nidus-avis* is free and the anther is versatile. Furthermore, the flowers are characterized by the absence of the abscission zone between perianth and ovary (a characteristic common to members of Vanilloideae; Pansarin & Barros 2008, Pansarin *et al.* 2008, 2012), rewardless, flowers opening in succession, with fleshy and indehiscent fruits (Fig. 3C), and ovoid seeds with a crustose seed coat (Figs. 3C-F). The seeds of *Pogoniopsis* possess characteristics also found in *Vanilla* and *Palmorchis*. Longitudinal cuts of the seeds and ovules of *Pogoniopsis* reveal the cells possess large nuclei and densely stained cytoplasm. The cytoplasm possesses granules, related to embryo nutrition (E.R. Pansarin, unpubl. data; Figs. 3E-F).

Reproductive biology within Pogonieae. Floral biology and pollination systems of Pogonieae is currently well known, since all genera of this subtribe has been studied on this subject. Among the North American-Eastern Asiatic clade data are available for *Pogonia ophioglossoides* (Thien & Marcks 1972)*, Pogonia japonica* (Matsui *et al.* 2001), *Isotria verticillata* and *I. medeoloides* (Mehrhoff 1983), in addition to *Cleistesiopsis divaricata* (syn. *Cleistes divaricata*) and *Cleistesiopsis bifaria* (syn. *Cleistes bifaria*; Gregg 1989, 1991a, 1991b). *Cleistesiopsis bifaria* and *C. divaricata* are pollinated by workers of *Bombus* and *Megachile* (Gregg 1989, 1991a, 1991b). *Isotria verticillata* is pollinated by small Halictidae, Anthophoridae and Andrenidae bees, while *I. medeoloides* is automatically self-pollinated (Mehrhoff 1983). *Pogonia ophioglossoides* is pollinated by queens and workers of bumblebees (Thien & Marcks 1972).

Pollination data regarding South-Central American clade (*i.e.,* genus *Cleistes*) are available to several species, including *C. libonii* (syn. *Cleistes macrantha*; Fig. 4A), *C. aphylla* (Fig. 4B)*, C. exilis* (Fig. 4C), *C. pusilla, C. paranaensis* (Fig. 4D), *C. bella* (Fig. 4E) and *C. rosea* (Fig. 4F) (Pansarin 2003; Pansarin *et al.* 2012). The species are pollinated by solitary or social bees, except *Cleistes libonii* (Fig. 4A), which is co-pollinated by hermit hummingbirds (Pansarin 2003). According to Pansarin *et al.* (2012), the reproductive phenology, the flowering strategies and the pollination mechanisms are similar among Brazilian *Cleistes*. In the spring (from October to November), each plant produces one unbranched aerial stem, with one erect and terminal raceme. Flowering generally occurs two or three months later, in the rainy season, from late December to early April. During a single flowering period, the species of *Cleistes* generally have three or four blooming peaks, each showing a precise synchronism in which all mature buds of most individuals within a population open simultaneously in the early morning. Flowering synchronism, as observed in all studied species of *Cleistes,* also has been recorded for basal Epidendroids, such as *Triphora trianthophora* (Medley 1979) and *Psilochilus modestus* (Pansarin & Amaral 2008).

The flowers of *Cleistes* possess two nectar glands at the base of the labellum, and a longitudinal and central crest with clusters of yellow or white colored hairs or papillae on the upper third, which act as a nectar guide. The column is parallel-disposed to the lip and presents a terminal versatile anther with two yellow clusters of free monads. The stigmatic surface is generally flat and possesses small and hyaline papillae. All studied species of *Cleistes* offer nectar as reward, which is produced by two nectar glands located on the base of the labellum. These glands are densely vascularized and the nectar is secreted along the nectary surface (Pansarin *et al.* 2012).

Duckeella adolphii possess lateral inflorescences with yellow flowers. The perianth parts are membranous. The basal portion of the labellum possesses a central crest covered by multicellular papillae. The elaiophor-like papillae are constituted by non-secretory cells.

Species of *Cleistes* and *D. adolphii* are dependent on pollen vectors for fruit setting. Coherent with the similarity among the floral morphology of the *Cleistes* flowers, the pollination mechanism is similar among *Cleistes*. The bees land on the lip apex and probe for nectar at its base. While the bees leave the flower, the anther is disarticulated with the scutellum and free pollen monads are placed on the scutum. Afterwards, the anther returns to the original position. In *Duckeella adolphii* the bees land directly on the central crest of the labellum, *i.e.,* on the elaiophor-like calluses (Pansarin 2003; Pansarin *et al.* 2012). Although the


A
B
C
D
E
F

presence of nectaries has been recorded at the base of the lip of *Cleistes* (Pansarin 2003. Pansarin *et al.* 2012), *Cleistesiopsis divaricata* and *Isotria verticillata* are pollinated by food deception, although both species bear nectar guides (Mehrhoff 1983; Gregg 1991a) and floral glands have been reported at the lip base of *Isotria verticillata* (Correll 1950). A hair-like cluster on the lip simulates pollen and attracts pollinators by deceit to the flowers of *Pogonia* (Dressler 1993) and *Cleistesiopsis bifaria* (Gregg 1989).

Like the North American genera *Cleistesiopsis*, *Pogonia* and *Isotria verticillata* (Thien & Marcks 1972, Mehrhoff 1983, Gregg 1989, 1991a), the South American *Cleistes* are pollinated exclusively by bees (Pansarin 2003, Pansarin *et al.* 2012). Visitation only on the first day of flower opening, even in species with longer lasting flowers, is probably related to the accumulation of the total volume of nectar at this time, no nectar production taking place afterwards. An exception is *C. libonii* whose flowers were visited also on the second day of anthesis (Pansarin 2003).

In Pogonieae the pollen is free and the pollen mass from a single flower, deposited on the scutum of a bee, may pollinate several flowers (Gregg 1991a, 1991b). Furthermore, some authors agree that flowering synchronism combined with the production of short-lived flowers may favor cross-pollination within a population (Medley 1979, Pansarin & Amaral 2008). According to Catling and Catling (1991), this kind of synchrony is an important strategy favoring cross-pollination in plants with one or few-flowered inflorescences. In fact, when all flowers in a population open simultaneously (*i.e.,* gregarious plants) the floral display for pollinator attraction is maximized (Pansarin *et al.* 2012).

The genus *Isotria* with floral glands at the lip base (Correll 1950, Cameron 2003) appears as sister to the remaining North American and Asiatic Pogonieae in some phylogenetic analyses based on chloroplast DNA (Pansarin *et al.* 2008). In the Amazonian *Duckeella adolphii* and in all other North American and Asiatic species (*Pogonia, Cleistesiopsis divaricata* and *C. bifaria*), floral glands are absent (see Thien & Marcks 1972, Gregg, 1989, 1991a, 1991b, Matsui *et al.* 2001). It has been suggested that in Orchidaceae deceptive mechanisms evolved from reward pollination systems (Dafni 1984, Ackerman 1986, Nilsson 1992). In the case of Pogonieae, reward production could be tested on the basis of molecular phylogeny. Our data suggest that ancestors of Pogonieae gave rise to two lineages, one of them spreading into tropical America, eventually originating the extant Neotropical *Cleistes* species with nectariferous flowers, and another one predominantly North American-Asiatic, but including the Amazonian *Duckeella*, pollinated through deceptive mechanisms (Fig. 1).

Some author argue that nectar can be energetically expensive and resources for reward production could be allocated more usefully for other functions capable of increasing fitness in species pollinated through deception (Boyden 1982, Ackerman 1986). The main problem with this hypothesis is that in many orchids, fitness is pollination limited, rather than resource limited (Calvo & Horvitz 1990, Calvo 1993). Furthermore, although few studies have been performed on this subject (see Pyke 1991), the rate of nectar production tends to be lower in short-lived than in long-lived flowers (Johnson & Nilsson 1999). Coherently, flowers in nectar-producing tropical *Cleistes* are shorter lasting than in the deceptive *Isotria, Pogonia*, *Cleistesiopsis divaricata* and *C. bifaria* (Thien & Marcks 1972, Mehrhoff 1983, Gregg, 1989, 1991b). On the other hand, in comparison with mechanisms involving nectar production, rewardlessness may reduce the visitation frequency, consequently lowering the reproductive success (Dafni 1984, Ackerman 1986, Johnson & Nilsson 1999, Johnson, 2000). Another consequence of deceptive mechanisms may be the reduction of natural fruit set and thus fruits are formed through geitonogamy

Left, Figure 4. A-F. Species of Pogonieae and its pollinators. A. *Bombus atratus* probing for nectar at the lip base of *Cleistes libonii.* The detail shows the bee leaving a flower. Note some pollen on its scutum; B. *Dialictus* sp. leaving a flower of *C. aphylla.* Note the pollen on its scutum. The detail shows the bee probing for nectar at the lip base; C. *Augochlora* sp. leaving a flower of *C. exilis*. Note the pollen deposited on its scutum. The detail shows the bee probing for nectar at the lip base; D. Flower of *C. paranaensis*; E. Flower of *C. bella* in diagonal view showing the white hairs on the central labellar crest; F. *Xylocopa* sp. with its scutum covered with a pollen load leaving a flower of *C. rosea*. The detail is a *Xylocopa* sp. probing for nectar at the lip base.

(Ackerman 1986, Johnson & Nilsson 1999, Johnson 2000; Smithson 2002, Pansarin & Pansarin 2014a). These claims are supported by comparing plants from reward-producing and deceptive Pogonieae clades (Pansarin *et al.* 2012). North-American Pogonieae have low reproductive success (Thien & Marcks, 1972, Mehrhoff 1983, Gregg 1989, 1991b), comparing to Brazilian species (E.R. Pansarin, unpubl. data). In certain years, fruit set of some species of *Cleistes* (*e.g., C. rosea, C. libonii, C. metallina,* and *C. ramboi*) reached nearly 100% (E.R. Pansarin, unpubl. data). It is worth mentioning that production of one to few short-lived flowers by each inflorescence of Central and South American *Cleistes* and the precise flowering synchronization tend to reduce the occurrence of self-pollination and geitonogamy (Pansarin *et al.* 2012). Furthermore, orchids with soft pollen (such as occurs in all Pogonieae species; Cameron & Chase 1999, Pansarin *et al.* 2008) apparently are not prone to fruit set through geitonogamous self- (Johnson & Nilsson 1999). Self-pollination resulting from geitonogamy is considered a mechanism of fitness reduction because of depression by inbreeding and pollen loss.

Reproductive biology within Vanilleae. Data on reproductive biology available on Vanilleae are scarce. Studies on pollination biology within Vanilleae have been performed predominantly on species of *Vanilla* (*e.g.,* Lubinsky *et al.,* 2006, 2010; Pansarin & Pansarin, 2014b; Pansarin *et al.,* 2014), which is the most diverse and economically important genus within Vanilloideae (Correll, 1953). The pollination system of *Epistephium* has been investigated more recently (Pansarin & Pansarin, 2014b). Within Vanilleae, euglossine bees have been recorded as pollinators of species of *Vanilla* (*i.e., V. planifolia* group and *V. pompona* group) in the Neotropics (*e.g.,* Lubinsky *et al.,* 2006). Euglossine bees are also the main pollinators of *Epistephium sclerophyllum* and *V. dubia* in Brazil (Pansarin *et al.,* 2014). In species of the *V. pompona* group, these bees are attracted by the fragrance of the flowers. They then display the typical behavior of pollination by male euglossine bees. Hummingbirds have been reported to pollinate some species of *Vanilla* (Bouriquet, 1954), while bats have been assumed to pollinate *V. chamissonis*, although this affirmation has been contested (Fleming *et al.,* 2009).

Vanilla insignis, *V. odorata* and *V. planifolia* are known to be pollinated through food deception (Soto Arenas *et al.,* unpubl. data). Due to low fruit set in natural populations, cultivated plants of *V. planifolia* are self-pollinated by hand to increase the production of fruits (Soto Arenas 1999). Some species of *Vanilla* yield fruits through spontaneous self-pollination (van der Pijl & Dodson 1966). This form of autogamy has been reported for *V. griffithii*, *V. palmarum*, populations of *V. planifolia*, and *V. savannarum* (see Pridgeon *et al.* 2003). Furthermore, flowers of a population of *V. bicolor* occurring in the Peruvian Amazon are obligatorily autogamous, producing fruits by cleistogamy (Van Dam *et al.* 2010).

Another Brazilian species that has been studied is *V. edwallii* (Fig. 5A), which is pollinated by *Epicharis* (Apidae: Centridini). *Vanilla edwallii* is rewardless, but male *Epicharis* are attracted to its flowers through the fragrance production (Pansarin *et al.* 2014). Additionally, Ruschi (1986) reports that *V. chamissonis* is visited by hummingbirds. However, in another study on this species, no pollinators were recorded, although fruits were observed in natural conditions (Reis *et al.* 2011). The flower morphology of *Vanilla* is very diverse, suggesting that different biotic vectors can act as pollinators of its species. Most species of *Vanilla* have tubular flowers where pollinators must enter through the tube formed by perianth and column (Dressler 1981). As occurs in *Vanilla*, the labellum margins of *Epistephium* are adnate to the column, resulting in a tubular flower (Dressler 1993).

Right, FIGURE 5. Species of Vanilleae and its pollinators. A. Flower of *Vanilla edwallii*. The detail shows a male *Epicharis* with pollen of *V. edwallii* on scutellum (arrow); B. Flower of *V. dubia*. The detail shows the cluster of penicillate hairs on the labellum (arrow); C. Labellum of *Epistephium sclerophyllym* in longitudinal section showing a *Bombus* (posed). Note it scutum just below the stigma and the articulated anther (arrow). The detail shows the cluster of hairs on the mid portion of the labellum (arrow); D. Labellum of *Epistephium sclerophyllym* in longitudinal section showing a bumblebee (posed). Note anther is disarticulated when the bee leaves the flower (arrow); E. *Plebeia droryana* collecting pollen directly from the anther. Note the corbiculae with pollen of the studied species; F. *Iridopelma* spider, capturing a pollinator (euglossine bee *Eufriesea nigrohirta*) on a flower of *E. sclerophyllum*.


A
B
C
D
E
F

Studies on the secretory tissues of Vanilleae are very scarce. In the rewardless *V. edwallii*, the only secretory tissues are osmophores located on labellum (Pansarin *et al.* 2014). In *V. edwallii,* the labellar trichomes produce a heterogeneous substance formed by mucilage and volatile compounds in the mentum region, which is associated with pollinator attraction (Pansarin *et al.* 2014). In this species the fragrance is produced by multicellular epidermal papillae in the median portion of labellum, between the lateral lobes and the entrance of the mentum region (Pansarin *et al.* 2014). In *Vanilla dubia*, the trichomes at the labellum base also secrete volatile substances, which are associated with pollinator attraction (Pansarin & Pansarin 2014b). Besides the oil droplets responsible for the odor of *Vanilla dubia*, starch grains were also observed within the labellar trichomes (Pansarin & Pansarin 2014b). Starch is a polysaccharide stored by plant cells as an energy source (Gonzáles 1999). In contrast to that recorded for *V. edwallii* (Pansarin *et al.* 2014), in *V. dubia*, starch grains are exclusively present on the labellum trichomes (Pansarin & Pansarin 2014b). Starch grains were already reported in association with osmophores in other Orchidaceae (Davies & Turner 2004), but it is commonly associated with the production of mucilage in different plant organs, including orchids (Leitão & Cortelazzo 2008).

Vanilla dubia is included in the *Vanilla planifolia* group, which includes vanillas with greenish flowers and a concave labellum with small papillae. The South American taxa of this group present a penicillate callus at the median portion of their labellum (Fig. 5B; Soto Arenas & Cribb 2010). Within this group, in addition to *V. dubia*, food deception has also been reported in *V. insignis*, *V. odorata* and *V. planifolia* (Soto Arenas *et al.,* unpubl. data). Pollination by male euglossine bees also has been reported for species of *Vanilla hostmanii* group: *V. cribbiana*, *V. dressleri V. hameri*; *Vanilla pompona* group: *V. pompona*, and *Vanilla trigonocarpa* group: *V. trigonocarpa* (Soto Arenas 1999, Pridgeon *et al.* 2003, Lubinsky *et al.* 2006, Soto Arenas & Dressler 2010), although the mechanisms involving fragrance collection are still unknown (Rodolphe *et al.* 2011). In *V. edwallii*, although fragrance is related to attracting male *Epicharis* (Apidae: Centridini) patrolling the flowers and maintaining them nearby, it is not a harvestable resource. *Vanilla edwallii* is a species of the *Vanilla parvifolia* group, which includes the reticulate-veined vanillas (Soto Arenas & Cribb 2010). *Vanilla inodora* (*Vanilla mexicana* group) seems to be pollinated by *Xylocopa* spp., although no evidence of pollination by these carpenter bees has been presented (Soto Arenas & Dressler 2010). An unidentified species of *Xylocopa* was also recorded on *V. edwallii*, but it only acted as a floral visitor, perforating the base of the labellum in search for nectar (Pansarin *et al.* 2014).

The nectaries of *Epistephium sclerophyllum* do not present differentiated structures, as occurs in genus *Cleistes*, whose nectar is produced by nectar glands (Pansarin 2003, Pansarin *et al.* 2012). Rather, they are flat and composed by a single-layered epidermis with rectangular cells and three or four layers of (rectangular) parenchymal cells, supplied by vascular bundles, like in other orchids (Stpiczyńska & Davies 2006). The similarity between the epidermal cells and the underlying parenchyma results in a nectary with a homogeneous pattern.

As recorded for *Vanilla edwallii* (Pansarin *et al.* 2014), Centridini bees (male *Centris*) were also observed patrolling the open flowers of *Epistephium sclerophyllum* waiting for females to mate. However, the flowers of *E. sclerophyllum* produce a minimal quantity of diluted nectar, which is probed by several bee species (Fig. 5C-F). In addition to nectar, pollen was directly collected from anthers by Meliponini bees (Fig. 5E; Pansarin & Pansarin 2014b). Since, in most Orchidaceae, pollen is aggregated and forms indivisible pollinia (*e.g.,* Dressler 1993), the offering of pollen as a resource is rare among orchids. The flowers of *Episthepium sclerophyllum* are scentless and bee attraction seems to be essentially visual, while the fragrance of the flowers of *Vanilla dubia* attracts male euglossine. Despite the presence of osmophores, the bees entering into the labellar tube appeared to be interested in nectar, not fragrances (Figs. 5C-D; Pansarin & Pansarin 2014b). The pollination mechanism in *Epistephium* is similar to that recorded in *Cleistes* (Pansarin 2003, Pansarin *et al.* 2012, Pansarin & Pansarin 2014b). The bees land on the labellum apex and probe for nectar at its base (Fig. 5C). As the bees leave the flower, the anther is disarticulated with the scutellum and free pollen monads placed on the thorax (Fig. 5D). Afterwards, the anther returns to the original position (Fig. 5C). Although pollination by other bee groups can occur (*E. sclerophyllum*; Figs. 5C-F), the flowers of both studied species are adapted to pollination by euglossines (Fig. 5F). Within Vanilleae, data are still inconclusive, but *Epistephium*, which, as recorded here for *E. sclerophyllum*, has nectariferous flowers, emerges as a sister to the rest of tribe. Within the monophyletic genus *Vanilla*, although more data on pollination biology are still needed, deceptive and rewarding species have been recorded (see Pansarin *et al.* 2014), suggesting that shifts between rewarding and rewardless species have happened more than once during the evolution of a genus (Fig. 1; Pansarin *et al.* 2012). This is reinforced by our recent observations, in the Amazonian forest, on the pollination biology of *Vanilla palmarum*, whose flowers produce nectar and are visited by hummingbirds (E.R. Pansarin and A.W.C. Ferreira, unpubl. data).

Although studies on vanilloid species pollinated by biotic vectors are scarce (exceptions in Lubinsky *et al.* 2006, 2010, Pansarin & Pansarin 2014b, Pansarin *et al.* 2014), spontaneous self-pollination has been recorded for some taxa, as *V. bicolor* (Van Dam *et al.* 2010), *Vanilla griffithii*, *V. palmarum* and *V. savannarum* (Pridgeon *et al.* 2003). Probably this is also the case with *Epistephium sclerophyllum*, whose stigmatic surface is not clearly separated from the anther (Pansarin & Pansarin 2014b). In *E. sclerophyllum* despite the formation of fruits through autogamy, bees act as pollinators, thus contributing to the possible formation of fruits trough cross-pollination.

ACKNOWLEDGEMENTS. The author thanks Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (grants 00/11015-9 and 07/07445-7), and Conselho Nacional do Desenvolvimento Científico e Tecnológico (grant 454490/2015-1) for funds provision.

LITERATURE CITED

Ackerman, J. D. (1986). Mechanisms and evolution of food-deceptive pollination systems in orchids. *Lindleyana* 1, 108–113.

Bouriquet, G. (1954). Culture. In: G. Bouriquet (Ed.). *Le vanillier et la vanille dans le monde* (pp. 430–458). Paris: Paul Lechevalier.

Boyden, T. C. (1982). The pollination biology of *Calypso bulbosa* var. *americana* (Orchidaceae): initial deception of bumblebee visitors. *Oecologia* 55, 178–184.

Calvo, R. N. & Horvitz, C. C. (1990). Pollinator limitation, cost of reproduction, and fitness in plants, a transition matrix demographic approach. *The American Naturalist* 136, 499–516.

Cameron, K. M. (2003). Vanilloideae. In: A. Pridgeon, P. Cribb, M. W. Chase & F. Rasmussen (Eds.). *Genera Orchidacearum* (pp. 281–334). Oxford: Oxford University Press.

Cameron, K. M. (2009). On the value of nuclear and mitochondrial gene sequences for reconstructing the phylogeny of vanilloid orchids (Vanilloideae, Orchidaceae). *Annals of Botany* 104, 377–385.

Cameron, K. M. & Chase, M. W. (1999). Phylogenetic relationships of Pogoniinae (Vanilloideae, Orchidaceae): an herbaceous example of the eastern North America – eastern Asia phytogeographic disjunction. *Journal of Plant Research* 112, 317–329.

Cameron, K. M., Chase, M. W., Whitten, W. M., Kores, P. J., Jarrell, D. C., Albert, V. A., Yukawa, T., Hills, H. G. & Goldman, D. H. (1999). A phylogenetic analysis of the Orchidaceae: evidence from *rbc*L nucleotide sequences. *American Journal of Botany* 86, 208–224.

Cameron, K. & Molina, M. C. (2006). Photosystem II gene sequences of *psbB* and *psbC* clarify the phylogenetic position of *Vanilla* (Vanilloideae, Orchidaceae). *Cladistics* 22, 239–248.

Catling, P. M. & Catling, V. R. (1991). A synopsis of breeding systems and pollination in North American orchids. *Lindleyana* 6, 187–210.

Correll, D. S. (1950). *Native orchids of North America*. Waltham: Chronica Botanica.

Correll, D. S. (1953). *Vanilla*- its history, cultivation and economic importance. *Economic Botany* **7**, 291–358.

Dafni, A. (1984). Mimicry and deception in pollination. *Annual Review of Ecology and Systematics* 15, 259–278.

Darwin, C. (1892). *The various contrivances by which orchids are fertilized by insects*. 2ND ed. rev. (1st ed. 1862). New York: Appleton.

Dressler, R. L. (1981). *The orchids: natural history and classification.* Cambridge: Harvard University Press.

Dressler, R. L. (1993). *Phylogeny and Classification of the Orchid Family*. Portland: Dioscorides Press.

Fleming, T. H., Geiselman, C. & Kress, W. J. (2009). The evolution of bat pollination: a phylogenetic perspective. *Annals of Botany* 104, 1017–1043.

Davies, K. L. & Turner, M. P. (2004). Morphology of floral papillae in *Maxillaria* Ruiz & Pav. (Orchidaceae). *Annals of Botany* 93, 75–86.

Gonzáles, M. L. (1999). Polisacáridos. In: A. M. Del Fresno (Ed.). *Farmacognosia General* (pp. 335). Madrid: Ed. Síntesis.

Gregg, K. B. (1989). Reproductive biology of the orchid *Cleistes divaricata* (L.) Ames var. *bifaria* Fernald growing in a West Virginia meadow. *Castañea* 54, 57–78.

Gregg, K. B. (1991a). Reproductive strategy of *Cleistes divaricata* (Orchidaceae). *American Journal of Botany* 78, 350–360.

Gregg, K. B. (1991b). Defrauding the deceitful orchid: pollen collection by pollinators of *Cleistes divaricata* and *C. bifaria*. *Lindleyana* 6, 214–220.

Johnson, S. D. (2000). Batesian mimicry in the non-rewarding orchid *Disa pulchra*, and its consequences for pollinator behavior. *Biological Journal of the Linnean Society* 71, 119–132.

Johnson, S. D. & Nilsson, L. A. (1999). Pollen carryover, geitonogamy, and the evolution of deceptive pollination systems in orchids. *Ecology* 80, 2607–2619.

Leitão, C. A. E. & Cortelazzo, A. L. (2008). Structural and histochemical characterization of the colleters of *Rodriguezia venusta* (Orchidaceae). *Australian Journal of Botany* 56, 161–165.

Lubinsky, P., Van Dam, M. H. & Van Dam, A. R. (2006). Pollination of *Vanilla* and evolution in Orchidaceae. *Lindleyana* 75, 926–929.

Lubinsky, P., Romero-González, G. A., Heredia, S. M. & Zabel, S. (2010). Origins and patterns of vanilla cultivation in tropical America (1500–1900): no support for an independent domestication of vanilla in South America. In: D. Havkin-Frenkel & F. C. Belanger (Eds.). *Handbook of vanilla science and* technology (pp. 117-138). Oxford: Wiley-Blackwell.

Matsui, K., Ushimaru, T. & Fujita, N. (2001). Pollinator limitation in a deceptive orchid, *Pogonia japonica*, on a floating peat mat. *Plant Species Biology* 16, 231–235.

Medley, M. E. (1979). *Some aspects of the life story of Triphora trianthophora (Sw.) Rydb. (Three birds orchid) with reference to its pollination*. MD Thesis. St Andrews: Andrews University.

Mehrhoff, L. A. (1983). Pollination in the genus *Isotria* (Orchidaceae). *American Journal of Botany* 70, 1444–1453.

Nilsson, L. A. (1992). Orchid pollination biology. *Trends in Ecology & Evolution* 7, 255–259.

Pansarin, E. R. (2003). Biologia floral de *Cleistes macrantha* (Barb. Rodr.) Schltr. (Orchidaceae: Vanilloideae: Pogoniinae). *Revista Brasileira de Botânica* 26, 73–80.

Pansarin, E. R. (2010a). *Vanilla dietschiana*, returns from *Dictyophyllaria*. *Orchids* 79, 106–109.

Pansarin, E. R. (2010b). Taxonomic notes on Vanilleae (Orchidaceae: Vanilloideae): *Vanilla dietschiana,* a rare south American taxon transferred from *Dictyophyllaria*. *Selbyana* 30, 198–202.

Pansarin, E. R. & Amaral, M. C. E. (2008). Pollen and nectar as a reward in the basal epidendroid *Psilochilus modestus* (Orchidaceae: Triphoreae): A study of floral morphology, reproductive biology and pollination strategy. *Flora (Jena)* 203, 474–483.

Pansarin, E. R. & Barros, F. (2008). Taxonomic notes on Pogonieae (Orchidaceae): *Cleistesiopsis*, a new genus segregated from *Cleistes*, and description of two new South American species, *Cleistes batistana* and *C. elongata*. *Kew Bulletin* 63, 441–448.

Pansarin, E. R., Salatino, A. & Salatino, M. L. F. (2008). Phylogeny of South American Pogonieae (Orchidaceae, Vanilloideae) based on sequences of nuclear ribosomal (ITS) and chloroplast (*psaB, rbcL rps16*, and *trnL-F*) DNA with emphasis on *Cleistes* and discussion of biogeographic implications. *Organisms, Diversity and Evolution* 8, 171–181.

Pansarin, E. R., Salatino, A., Pansarin, L. M. & Sazima M. (2012). Pollination systems in Pogonieae (Orchidaceae: Vanilloideae): A hypothesis of evolution among reward and rewardless flowers. *Flora (Jena)* 207, 849–861.

Pansarin, E. R., Aguiar, J. M. R. B. V. & Pansarin, L. M. (2014). Floral biology and histochemical analysis of *Vanilla edwallii* Hoehne (Orchidaceae: Vanilloideae): an orchid pollinated by *Epicharis* (Apidae: Centridini). *Plant Species Biology* 29, 242–252.

Pansarin E. R. & Pansarin L. M. (2014a). Reproductive biology of *Epidendrum tridactylum* (Orchidaceae: Epidendroideae): a reward-producing species and its deceptive flowers. *Plant Systematics and Evolution* 300, 321–328.

Pansarin, E. R. & Pansarin, L. M. (2014b). Floral biology of two Vanilloideae (Orchidaceae) primarily adapted to pollination by euglossine bees. *Plant Biology (Stuttgart)* 16, 1104–1113.

Pridgeon, A. M., Cribb, P. J., Chase, M. W. & Rasmussen, F. N. (2003). *Genera Orchidacearum, vol. 3, Orchidoideae (Part 2), Vanilloideae*. London: Oxford University Press.

Pyke, G. H. (1991). What does it cost a plant to produce floral nectar? *Nature* 350, 58–59.

Reis, C. A. M., Brondani, G. E. & Almeida, M. de (2011). Biologia floral e propagação vegetativa de baunilha. *Scientia Agraria Paranaensis* 10, 69–82.

Rodolphe, G., Séverine, B., Michel, G. & Pascale, B. (2011). Biodiversity and evolution in the *Vanilla* genus. In: O. Grillo & G. Venora (Eds.). *The dynamical processes of biodiversity - case studies of evolution and spatial distribution* (pp. 1–27). Rijeka: InTech.

Ruschi, A. (1986). *Aves do Brasil.* Rio de Janeiro: Expressão e Cultura.

Smithson, A. (2002). The consequences of rewardlessness in orchids: reward-supplementation experiments with *Anacamptis morio* (Orchidaceae). *American Journal of Botany* 89, 1579–1587.

Soto Arenas, M. A. (1999). *Filogeografía y recursos genéticos de las vanillas de México.* México

DF: Instituto Chinoin AC. Informe final SNIB-CONABIO proyecto No. J101.

Soto Arenas, M. A. & Cribb, P. (2010). A new infrageneric classification and synopsis of the genus *Vanilla* Plum. *ex* Mill. (Orchidaceae: Vanillinae). *Lankesteriana* 9, 355-398.

Soto Arenas, M. A. & Dressler, R. L. (2010). A revision of the Mexican and Central American species of *Vanilla* Plumier *ex* Miller with a characterization of their ITS region of the nuclear ribosomal DNA. *Lankesteriana* 9, 285–354.

Stpiczyńska, M. & Davies, K. L. (2006). Nectary structure in *Symphyglossum sanguineum* (Rchb.f.) Schltr. (Orchidaceae). *Acta Agrobotanica* 59, 7–16.

Thien, L. B. & Marcks, B. G. (1972). The floral biology of *Arethusa bulbosa*, *Calopogon tuberosus*, and *Pogonia ophioglossoides* (Orchidaceae). *Canadian Journal of Botany* 50, 2319–2325.

Van Dam, A. R., Householder, J. E. & Lubinsky, P. (2010). *Vanilla bicolor* Lindl. (Orchidaceae) from the Peruvian Amazon: auto-fertilization in *Vanilla* and notes on floral phenology. *Genetic Resources and Crop Evolution* 57, 473–480.

van der Pijl, L. & Dodson, C. H. (1966). *Orchid flowers, their pollination and evolution.* Florida: University of Miami Press.

LANKESTERIANA 16(2): 269–278. 2016. **doi**: http://dx.doi.org/10.15517/lank.v16i2.26011

EFECTO DEL HERBICIDA GLIFOSATO EN HONGOS ENDÓFITOS DE RAÍZ Y KEIKIS DE *EPIDENDRUM MELINANTHUM* (ORCHIDACEAE)

ERIKA PEREA-MORERA[1,2,5] & JOEL TUPAC OTERO[2,3,4]

[1] Facultad de Ingeniería y administración, Universidad Nacional de Colombia sede Palmira.
Carrera 32 No 12-00 Chapinero, Vía Candelaria. Palmira, Valle del Cauca, Colombia

[2] Grupo de Investigación en Orquídeas, Ecología y Sistemática Vegetal; Departamento de Ciencias Biológicas, Universidad Nacional de Colombia Sede Palmira Calle 32 N° 12-00,
Palmira-Valle del Cauca-Colombia

[3] Departamento de Ciencias Biologicas, Universidad Nacional de Colombia sede Palmira.
Carrera 32 No 12-00 Chapinero, Vía Candelaria. Palmira, Valle del Cauca, Colombia

[4] Instituto de Ciencias Ambientales IDEA, Universidad Nacional de Colombia Sede Palmira

[5] Author for correspondence: epeream@unal.edu.co

RESUMEN. El uso de herbicidas ha tenido un impacto grande sobre la producción agrícola y sin duda ha aumentado la producción en muchas cosechas. Desafortunadamente, la aplicación de herbicidas puede tener efectos negativos sobre otras especies nativas aledañas al cultivo. Con el fin de establecer si el herbicida glifosato afecta negativamente a la orquídea terrestre *Epidendrum melinanthum* y sus hongos endófitos aislados de la raíz, se evaluaron tres dosis de Round Up® en keikis y en hongos endófitos previamente aislados. Las dosis de aplicación se determinaron a partir de la utilizada para la maduración en caña de azúcar (1.5 l/Ha equivalentes a 544.5 g de glifosato/l) de la siguiente manera: a) control (sin herbicida), b) dosis de aplicación en caña de un décimo y c) una centésima parte de la dosis de maduración. Se observó en keikis el cambio de coloración en tallo y en hojas, adicional a la caída de las mismas. El Round Up generó afectación en uno de los tres hongos estudiados en forma de un halo de inhibición. Se concluye, que el glifosato afecta negativamente las estructuras foliares de los keikis de manera rápida al aplicar el herbicida sobre las hojas, en adición a la inhibición de hongos endófitos de *Epidendrum melinanthum*. Estos aspectos sugieren el potencial negativo del herbicida sobre la especie estudiada y su microflora asociada.

PALABRAS CLAVES: efectos del glifosato, *Epidendrum melinanthum*, inhibición de hongos, keikis, orquídeas, Round Up

Introducción. Los herbicidas son económicamente rentables para la erradicación de algunas malezas en cultivos productivos, especialmente en cultivos que son de lento crecimiento, pues en estos cultivos se favorece la aparición de hierbas que pueden llegar a reducir el rendimiento en un 60–70% (Hajebi *et al*. 2015). Los herbicidas pueden clasificarse de múltiples maneras, bien sea por la utilización, por las propiedades químicas y el modo de acción (Labrada *et al*. 1996) y pueden afectar a las plantas dependiendo de las características que cada especie tenga, la edad, la cantidad, las condiciones ambientales, y el tiempo transcurrido después de la aplicación (FAO 2016). Por lo tanto, existen especies que son resistentes a algunos herbicidas como es el caso de *Zea mays* L. y *Glycine max* L. que presentan resistencia al glifosato (Obour *et al*. 2016). Si bien los herbicidas juegan un importante papel en el control de malezas (Freitas *et al*. 2016) y como es el caso del glifosato, puede actuar como fuente de carbono, estimular la actividad microbiológica del suelo y la respiración microbiana (Obour *et al*. 2016). Pero el uso de herbicidas puede impactar negativamente los ecosistemas, acumularse en la cadena alimentaria y afectar gravemente la salud humana (Freitas *et al.* 2016).

Las especies de la familia Orchidaceae tienen grandes amenazas dado que son extremadamente susceptibles a cambios en su entorno en comparación con otras plantas, las afectan principalmente las malezas, agentes patógenos, cambio climático,

Received Received 9 May 2016; accepted for publication 12 August 2016. First published online: 30 August 2016.

incendios, animales, perdida de hábitat, prácticas agrícolas, el uso de fertilizantes y el turismo (Pillon *et al.* 2006, Ranking *et al.* 2015), por lo cual, tienen una mayor proporción de géneros y especies amenazadas que la mayoría de familias (Zhang *et al.* 2015). Dada la alta vulnerabilidad y la limitada distribución de la mayoría de las especies, efectos locales pueden resultar en extinciones de especies (Zhang *et al.* 2015). Los efectos negativos sobre las poblaciones de orquídeas en general se refieren a la deforestación o la sobre-explotación, pero hay efectos indirectos que podría estar causando la reducción en tamaño poblacional que son más sutiles. El uso de herbicidas en grandes extensiones como la producción de caña de azúcar podría tener un impacto en la flora que rodea estas áreas agrícolas, por lo tanto, es imperativo realizar un análisis de los impactos negativos generados por prácticas agrícolas, esto con el fin de diseñar políticas de conservación.

El Valle del Río Cauca se caracteriza por ser la zona donde se encuentra ubicado el principal sector azucarero de Colombia, el cual cuenta con aproximadamente 225,560 Ha de tierra sembradas en caña (ASOCAÑA 2012), dentro del proceso de cultivo de la caña de azúcar se encuentra la maduración, proceso fisiológico en el cual se favorece la concentración de sacarosa en la caña (Cassalett *et al.* 1995). Desde 1920 se ha venido investigando sobre el uso de madurantes, siendo el glifosato el que presenta efectos más consistentes y de mayor efectividad para el cultivo de caña, pues aumenta el rendimiento de sacarosa en el cultivo, logrando el aumento de hasta cinco veces más de sacarosa en el tallo (Su *et al.* 1992, Cassalett *et al.* 1995), con dosis entre 1.0 y 2.0 l/Ha con aplicaciones entre la sexta y catorceava semana (Cassalett *et al.* 1995).

Los productos comerciales implementados para la maduración son el Round Up o glifosol, cuyo objetivo es concentrar en el tallo un mayor contenido de azúcar en la caña (Cassalett *et al.* 1995). El componente activo de los productos utilizados para la maduración es el glifosato (*N* – (fosfonometil) glicina), este es mayormente utilizado como un herbicida general y ha sido utilizado desde 1974 en numerosas presentaciones comerciales y distribuido a nivel mundial (Steinmann *et al.* 2012). La acción del glifosato se desencadena por la inhibición de la enzima 5-enolpiruvilshikimato-3-fosfato sintetasa (EPSPS), mediante la ruta del ácido shikímico que impide la síntesis de tres aminoácidos esenciales (fenilalanina, tirosina y triptófano), lo cual impide crecimiento e inhibe la capacidad de eliminar toxinas (Cox 1998). El glifosato en dosis de 1 kg/Ha es un herbicida no selectivo, además, es absorbido por las hojas de la planta y se transloca a las raíces, lo cual permite una actividad sistémica (Steinmann *et al.* 2012).

Beyrle *et al.* (1994) demostraron que la incidencia del glifosato inhibe la germinación de semillas de la orquídea terrestre de la zona templada *Orchis morio*, además, evalúan el efecto del glifosato sobre el crecimiento de algunos hongos micorrízicos y la inhibición de la iniciación micorrízica. Los objetivos de este estudio son: evaluar el efecto del glifosato sobre *Epidendrum melinanthum*, respecto al número, color y curvatura de hojas, así como la producción de keikis. Adicionalmente, se pretende determinar si hay un efecto del glifosato en el crecimiento de hongos endófitos.

Materiales y métodos

Epidendrum melinanthum Schltr. (Orchidaceae: Epidendroideae: Laeliinae) (Romero & Ferreira 2006) es una orquídea terrestre presente en bosque seco tropical que presenta óptimo crecimiento en franjas comprendidas entre 1100 y 1300 msnm, con inflorescencia racemosa, labelos divididos en tres lóbulos y color amarillo en sépalos y pétalos (Fig. 1) (Reina-Rodríguez & Otero 2011).

El estudio se realizó con *E. melinanthum* como especie modelo, dado que es una especie ampliamente distribuida en el Valle del Cauca, además de ser una especie que es vulnerable a las actividades agrícolas de la caña pues su hábitat está en zonas aledañas al monocultivo.

Material vegetal. Colección de raíces. Las raíces se colectaron en el corregimiento de Los Ceibos ubicado en el municipio de Palmira-Valle del Cauca el mes de noviembre del 2014. Las raíces se obtuvieron de plantas que se encontraron en buen estado y sin deteriorar su sistema radicular (Fig. 2). Las muestras se conservaron en bolsas de polietileno etiquetadas con el fin de mantener la humedad hasta ser procesadas en el laboratorio el mismo día (Mosquera *et al.* 2010, Han *et al.* 2016).

Figura 1. Flor de *Epidendrum melinanthum* en el Valle del Cauca.

Figura 2. Raíces de *Epidendrum melinanthum*.

Colección de keikis. Los keikis de *E. melinanthum* de alrededor de 10 cm, se obtuvieron de la población natural de La Vorágine-Cali y Tablones Palmira en noviembre del 2014. El material vegetal se depositó en bolsas de papel, se etiquetaron y se transportaron en una nevera de icopor hasta el laboratorio.

Aislamiento de hongos. Dos morfotipos de hongos endófitos fueron aislados de raíces de *E. melinanthum* y uno de papa. Cada raíz se cortó en secciones de 1 cm y se etiquetaron, se procedió a sembrar en agar de dextrosa de patata (PDA) dentro de la cámara de flujo laminar (Valadares *et al.* 2015), con antelación las raíces se esterilizaron superficialmente en etanol al 70% por 1 min, hipoclorito de sodio al 2.5% por 2 min, etanol al 70% por 1 min y se realizaron tres lavados con agua destilada estéril (Otero *et al*. 2002, Otero & Bayman 2009, Tan *et al.* 2014). Al azar se colocaron trozos de cada sección en PDA, se incubaron a 25°C y se observaron diariamente. Cada nueva hifa se transfirió a PDA para obtener los hongos purificados y su posterior identificación (Otero & Bayman 2009, Tan *et al.* 2014).

Caracterización morfológica de los hongos endófitos aislados. La caracterización morfológica de los tres hongos aislados se llevo a cabo mediante la observación de placas en el microscopio. Los hongos de *Rhizoctonia* presentan anamórfismos y telemórfismos, pero se caracterizan por tener un ángulo de ramificación recto, presentan hifas con constricción en tabiques, en ángulo recto ramificado de las hifas después de los tabiques y con frecuencia presentan hifas inflamadas bien conocidas como monillioid (Otero *et al.* 2002, Otero *et al.* 2004, Otero *et al.* 2005, Otero *et al.* 2007, Pereira *et al.* 2014).

Concentración de glifosato. Efecto del glifosato en el crecimiento de hongos endófitos. El efecto del glifosato sobre el crecimiento de hongos endófitos aislados se realizó de dos maneras, la primera consistió en poner un disco de papel filtro estéril con las dosis estudiadas de glifosato en cajas Petri que contenían PDA y se procedió a sembrar los hongos; se realizaron observaciones de la formación del halo de inhibición en las tres dosis y el control. La segunda prueba, consistió en disolver el glifosato en el PDA justo cuando se estuviera sirviendo el medio, esto con el fin de observar el diámetro de crecimiento de los tres morfotipos de hongos aislados, uno de papa y dos de *E. melinanthum*.

Efecto del glifosato sobre el número, color y curvatura de hojas y producción de keikis de *E. melinanthum*. Las plántulas se trasplantaron en macetas que contenían sustrato para orquídeas de contextura media adquirida en el orquideograma de Cali. Posteriormente, las macetas se aclimataron en el invernadero de la Universidad Nacional de Colombia Sede Palmira con humedad relativa de 70–80 %, aproximadamente de 12 h d^{-1} fotoperíodo, 120–180 mol m^{-2} s^{-1} intensidad de luz PPF (luz solar sombreada) y régimen de temperatura 28±2 °C durante el día y 25±2 °C durante la noche (Panwar *et al.* 2012). Posteriormente, los keikis se organizaron de manera homogénea y se dividieron con láminas de vidrios para que las dosis de glifosato aplicadas no se mezclaran.

Se realizó la aplicación de Round Up donde la dosis 1, es equivalente a 1.5 l/Ha (544.5 g/l), la dosis típicamente usada en la cosecha de caña de azúcar para la maduración, entonces el diseño incluye tres dosis experimentales 1, 1/10, 1/100 y el control con H_2O destilada estéril con 30 repeticiones cada una, se realizaron dos aplicaciones con una diferencia temporal de 15 días y se realizó seguimiento semanal al número de hojas, color de las hojas y tallos, curvatura de hojas y producción de keikis, durante seis semanas posterior a la primera aplicación.

Análisis histológico

Al final del experimento se realizaron cortes histológicos con el fin de evidenciar las diferencias en tallo y hojas, respecto al control. El análisis se realizó a través de cortes histológicos de aproximadamente 100 µm que fueron posteriormente teñidos con azul de toluidina y observados en el microscopio (Puerto *et al*. 2001).

Métodos estadísticos

Se realizó el análisis de varianza (ANOVA) con un nivel de significación de a=0.05. Además, se realizó una comparación de medias con la prueba de rango múltiple de Duncan para el análisis de la perdida de hojas en *E. melinanthum* y crecimiento de hongos endófitos, y por otra parte, se realizó un análisis de correspondencia múltiple cualitativa en el efecto de color, curvatura y producción de keikis; se efectuó mediante el paquete estadístico SAS (2000).

La prueba de rango múltiple de Duncan se realizó porque permite comparar todos los tratamientos entre sí, además, el número que se analizó no era profuso, por lo cual era ideal para obtener resultados que determinaran si existen diferencias significativas en la investigación. Paralelamente, se realizó la prueba de correspondencia múltiple cualitativa con el fin de observar en un mapa las variables estudiadas para identificar las asociaciones entre las mismas. Por consiguiente, la hipótesis nula (H_0) es: si el glifosato afecta negativamente el crecimiento de hongos endófitos de *E. melinanthum*, y en segundo lugar, la H_0 para la prueba de pérdida de hojas equivale a; si el glifosato favorece la perdida de hojas de *E. melinanthum*.

Resultados

Caracterización morfológica de los hongos endófitos aislados

Se obtuvieron tres hongos aislados determinados como *Rhizoctonia* sp., de los cuales, dos se obtuvieron de las raíces de *E. melinanthum*, y uno de papa. Todos los hongos aislados cumplían las características descritas en la metodología.

Efecto del glifosato en el crecimiento de hongos endófitos

Se obtuvo que el glifosato a la concentración 544.5 g/l inhibe significativamente el crecimiento del morfotipo (B) aislado de *E. melinanthum* con respecto a los hongos restantes, pues la probabilidad mayor que el F calculado ($Pr > F) = 0.0422 < 0.05$. El crecimiento en este morfotipo era más lento con respecto al control y forma un halo de inhibición a concentración 544.5 g/l (Fig. 3). Por otra parte, se observó que no hay diferencias significativas entre el control y las tres dosis de glifosato aplicadas, pues $Pr > F = 0.9673 > 0.05$, sin embargo, el 50% de los hongos, equivalente a la diferencia de los cuartiles tres y uno, crece con un diámetro menor en la concentración 544.5 g/l con respecto al control, evidenciando que existen diferencias significativas entre las dos concentraciones pues las áreas de las cajas y sus respectivos bigotes no se interceptan (Fig. 4), a la concentración 544.4 g/l de glifosato pueden llegar a afectar el crecimiento de los tres hongos aislados, como se observa en la Fig. 4, donde se muestran los diámetros medios de crecimiento de los hongos aislados.

Efecto del glifosato sobre el color, curvatura, producción de keikis y número de hojas de E. melinanthum

Se observaron daños en tallos y hojas de diversas sintomatologías, que a continuación se describen:

> El color de tallo y hojas: Se presentaron cambios de color en hojas en tonalidades de amarillo con manchas violetas (Fig. 5A), puntos y manchas violetas (Fig. 5B), puntos y manchas blancas (Fig. 5C y 5D), puntos negros (Fig. 5C), manchas con tonalidades café (Fig. 5C), en la zona de transición ente la hoja y el tallos se observa una tonalidad negra en los tejidos (Fig. 5D), por su parte, en los tallo se evidenció la aparición de manchas negras (Fig. 5D).

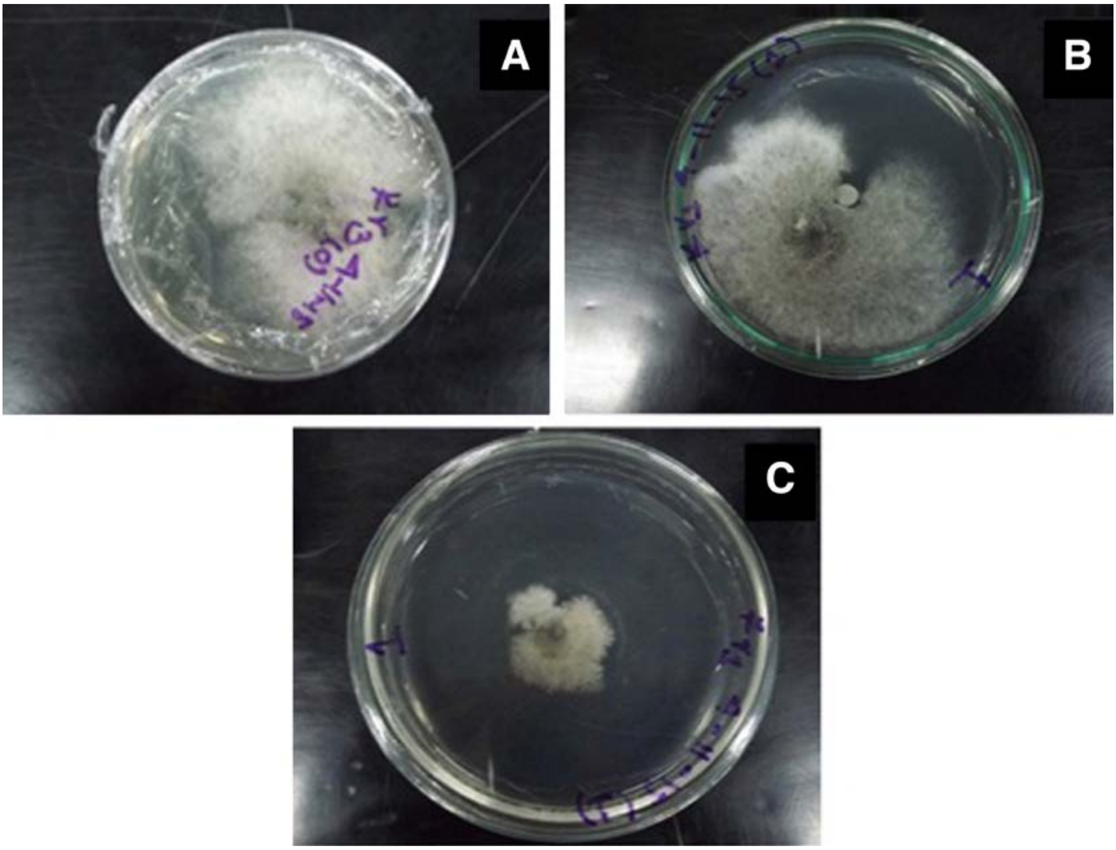


Figura 3. A. Hongo sembrado en PDA (control). B. Halo de inhibición por dosis 1 de glifosato. C. Disminución de crecimiento con dosis 1.

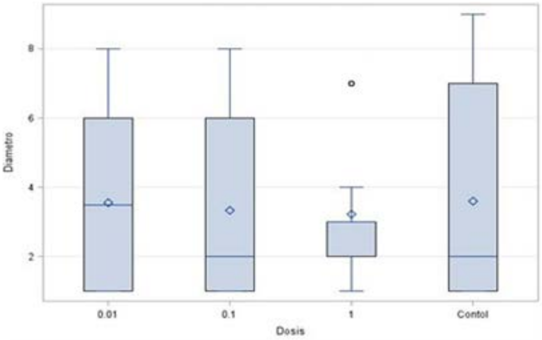


Figura 4. Distribución de diámetros promedio acuerdo a las dosis aplicadas. La línea del centro de la caja representa la mediana, el diamante representa la media y los bigotes representan los percentiles 5% - 25% y 75% - 95% y la caja representa el 50% equivalente a la resta del cuartil tres y cuartil uno. Los puntos que se encuentran por fuera de los bigotes son datos atípicos.

Curvatura de hojas: luego de la aplicación del glifosato se observo que las hojas se tornaron curveadas y cóncavas con diversas tonalidades (Fig. 5B).

Producción de keikis: se encontró que la producción de keikis era mayor en dosis 1/10, seguido de la concentración de 544.5 g/l equivalente a la dosis 1 comparados con el control (Fig. 6), sin embargo, la producción de keikis no es representativa. También, se presentan semejanzas entre los efectos obtenidos en keikis por las aplicaciones de Round Up, agrupadas junto con la curvatura de las hojas y la producción de plántulas, lo cual indica que dichos efectos son producidos por las aplicaciones de glifosato (Fig. 7). Se puede observar que en la dosis 0.1 hubo un considerable número de keikis al igual que en la dosis 1 equivalente a la concentración 544.5 g/l (Fig. 6).

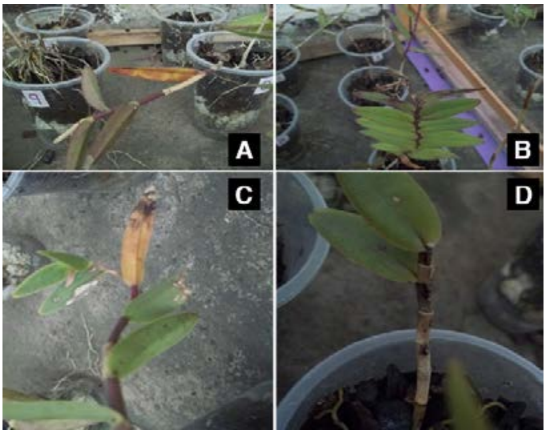


Figura 5. A. Hojas mareadas y amarillas. B. Hojas con puntos morados y curveadas. C. Hojas amarillas con café, con puntos negros y blancos. D. Tallo negro.

Número de hojas: se presentaron mayores pérdidas de hojas en las dosis 1 y 0.1 con respecto al control, al igual se presentan diferencias significativas entre las aplicaciones previas y posteriores a la aplicación del Round Up, pues Pr > F = 0.0002 < 0.05 (Fig. 8).

Los cortes histológicos a nivel microscópico de las afectaciones en hoja y tallo de *E. melinanthum* (Fig. 9), indicaron que en el tallo se afecta el haz vascular, dentro del cual, el xilema y el floema se ven deformados y quemados por el químico (Fig. 9D), por su parte, las hojas se encuentran afectadas principalmente la cutícula y la epidermis, pues presentan deformidades y pudrimiento en algunos sectores de las células (Fig. 9F, Fig. 9G y Fig. 9H).

Discusión

Los hongos aislados de las raíces de *E. melinanthum* se ven afectados por el glifosato con dosis aplicadas en la caña de azúcar. El efecto mayor se ve en la concentración de 544.5 g/l pero se evidencia que también tiene efecto en la dosis 1/10 con respecto al control. Este tipo de patrón se observó también en la perdida de hojas, pero a dichas concentraciones, el glifosato puede llegar a generar una considerable cantidad de keikis como un medio de respuesta para la supervivencia de la orquídea. Zhao *et al.* en 2014 detectaron el éxito que tiene el glifosato a la hora de dañar el cultivo pues las hojas recién nacidas que

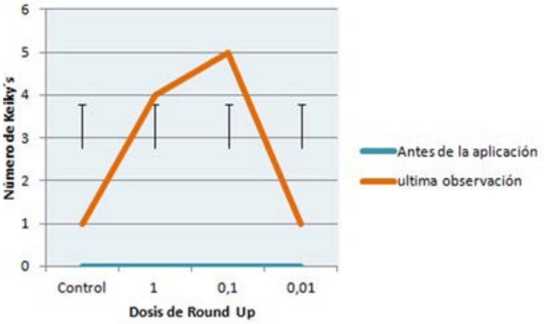


Figura 6. Producción de keikis antes y después de la aplicación de glifosato en las dosis estudiadas. Las barras representan el error con desviación estándar con tamaño de muestra de 30 plántulas por cada dosis.

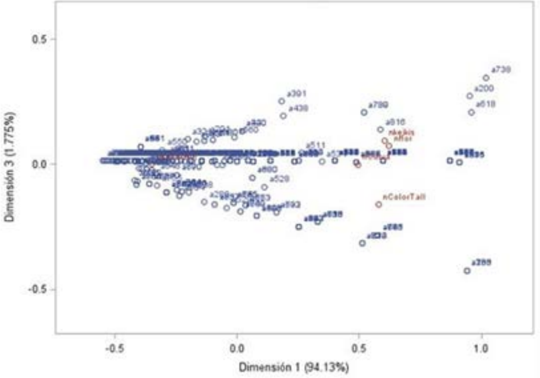


Figura 7. Semejanzas entre los efectos presentados en keikis, color de tallo, color de hojas y curvatura.

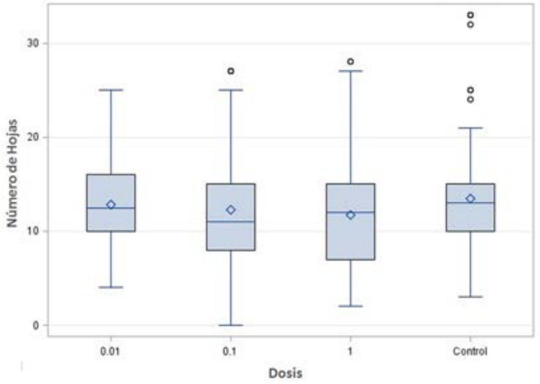


Figura 8. Pérdida de hojas por tratamiento durante el tiempo del estudio. La línea del centro de la caja representa la mediana, el diamante representa la media y los bigotes representan los percentiles 5% - 25% y 75% - 95% y la caja representa el 50% equivalente a la resta del cuartil tres y cuartil uno. Los puntos que se encuentran por fuera de los bigotes son datos atípicos

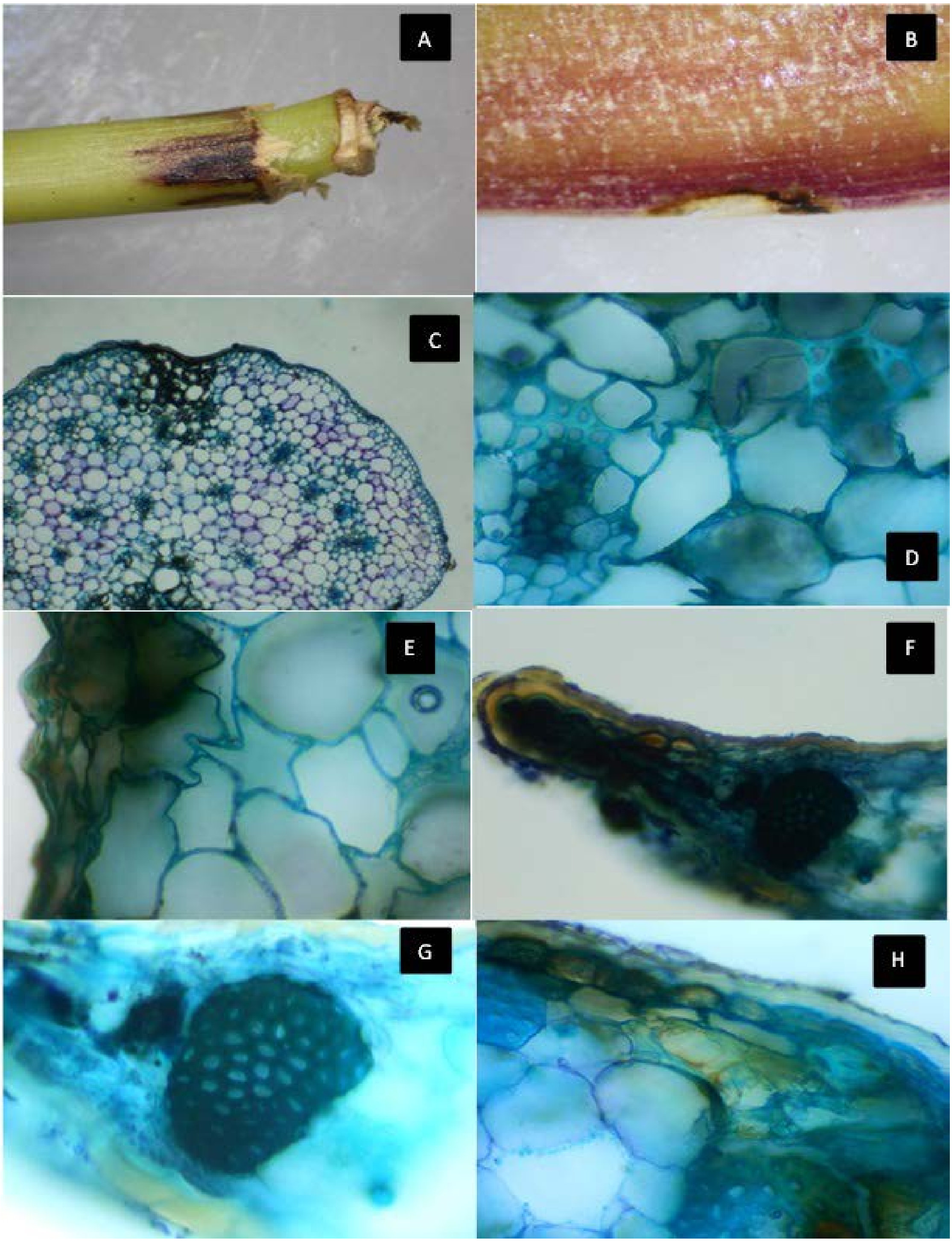


FIGURA 9. A. Segmento de tallo afectado. B. Segmento de hoja afectada. C. Efecto de glifosato en tallo a 4X. D. Afectación en haz vascular a 40X. E. Afectación en el velamen a 40X. F. Corte transversal de hoja donde se observa la quemazón y afectación en esclerénquima a 10X. G. Daño en Esclerénquima a 40X. H. Daño en cutícula y epidermis**.**

generalmente se encuentran en la zona superior de las plantas son más sensibles a la disminución de la clorofila, por otra parte obtuvo que la bioquímica foliar es un indicador potencialmente para la detección temprana del estrés que presentan las plantas a causa de las lesiones del glifosato, pues las lesiones se pueden detectar pasadas 48 horas de la aplicación.

Si bien el glifosato inhibe el crecimiento de unos hongos aislados de *E. melinanthum*, también puede reducir la viabilidad, la germinación y el crecimiento de las esporas de los hongos, así como la colonización de hongos micorrízicos, pues los hongos pueden verse afectados en zonas aledañas a la aplicación del herbicida e incluso cuando se ve impulsado por la deriva del viento (Druille *et al.* 2013). Otro tipo de afectación que puede ser aún más grave es que el glifosato puede llegar vía indirecta a los hongos, es decir, que el herbicida pude cambiar los suministros de hidratos de carbono (Druille *et al*. 2013), pues el glifosato puede llegar a reducir la fuente energética de la planta o a la destrucción de la pared celular de la orquídea conduciendo de esta manera a la muerte de la planta.

Dosis altas de glifosato afectan principalmente las hojas de *E. melinanthum* que se evidencian en el cambio de coloración, la caída de las hojas, la inhibición de la fotosíntesis, lo que puede conllevar a la muerte la planta. Dado que, el glifosato impacta la tasa fotosintética pues reduce el contenido de clorofila en las hojas cuando se aplica en el follaje de las plantas (Druille *et al*. 2013), pues los fotorreceptores situados en la clorofila son los encargados de absorber de manera eficaz la luz azul-violeta (450–500 nm) y rojo-naranja (650–750 nm), pues la fotosíntesis es más eficiente para las plantas en la longitud de onda citada (Gliessman 2002). Dado lo anterior, se puede observar las diversas sintomatologías causadas por el glifosato en las hojas produciendo una marchitez que termina en la perdida de las hojas o privando la respiración a la planta.

El Round Up aplicado no actúa sistémicamente en las orquídeas, pues lo que genera son afectaciones localizadas, por lo que se considera un agroquímico contacto cuando actúa a dosis altas en *E. melinanthum,* afectando negativamente el velamen en el tallo, la cutícula, el haz vascular y la epidermis en hojas, lo cual puede ocasionar desecación de las hojas y desprotección de la orquídea a los ataques de bacterias y hongos (Boom *et al.* 2005). Se puede deducir que se presenta curvatura en las hojas de la orquídea porque detiene el crecimiento de algunas células, lo que se visualiza puntualmente en la distorsión de la forma normal de la hoja.

Literatura citada

ASOCAÑA, (2012). El sector azucarero en la actualidad. http://www.asocana.org/publico/info.aspx?Cid=215; Consulta: febrero 2015.

Beyrle, H. F., Smith, S. E., Franco, C. M. M., & Peterson, R. L. (1995). Colonization of *Orchis morio* protocorms by a mycorrhizal fungus: effects of nitrogen nutrition and glyphosate in modifying the responses. *Canadian Journal of Botany*, 73(8), 1128–1140. DOI: 10.1139/b95-123

Boom, A., Sinninge, J. S., De Leeuw, J. W. (2005). Cutan, a common aliphatic biopolymer in cuticles of drought-adapted plants. *Organic Geochemistry*, 36 (4), 595–601. DOI: 10.1016/j.orggeochem.2004.10.017

Cassalett, D. C., Torres, A. J., Isaacs, E. C. (1995). El cultivo de la caña en la zona azucarera de Colombia. 1th ed. Cali, Cenicaña, 394 p.

Cox, C. (1998). Glyphosate (Round up). *Journal of Pesticide Reform*, 18(3), 3-15.

Druille, M., Omacini, M., Golluscio, R. A., Cabello, M. N. (2013). Arbuscular mycorrhizal fungi are directly and indirectly affected by glyphosate application. *Applied Soil Ecology*, 72, 143–149. DOI: 10.1016/j.apsoil.2013.06.011

FAO. (2016). Herbicide action. (Consultado: 1 de julio de 2016, http://www.fao.org/ag/agp/agpc/doc/Publicat/FAOBUL2/B206.htm).

Freitas, M. R., Barigye, S. J., Daré, J. K., Freitas, M. (2015). Aug-MIA-SPR/PLS-DA classification of carbonyl herbicides according to levels of soil sorption. *Geoderma,* 268, 1–6. DOI: 10.1016/j.geoderma.2016.01.013

Gliessman, S. R. (2002). Introducción. En: Agroecología. En: Procesos ecológicos en agricultura sostenible (pp. 385). Costa Rica: LITOCAT.

Han, J. Y., Xiao, H., Jiangyun, G. (2016). Seasonal dynamics of mycorrhizal fungi in *Paphiopedilum spicerianum* (Rchb. f) Pfitzer — A critically endangered orchid from China. *Global Ecology and Conservation*, 6, 327–338. DOI: 10.1016/j.gecco.2016.03.011

Hajebi, A., Das, T. K., Arora, A., Bala, S., Hajebi, F. (2015). Herbicides tank-mixes effects on weeds and productivity and profitability of chilli (*Capsicum annuum* L.) under conventional and zero tillage. *Scientia Horticulturae,* 198, 191–196. DOI: 10.1016/j.scienta.2015.11.020

Landra, R., Caseley, J. C., Parker, C. (1996). Herbicidas. Pp.193–195 en: Manejo de Malezas para Países en Desarrollo (Estudio FAO Producción y Protección Vegetal - 120). Roma: FAO.

Mosquera-Espinosa, A.T., Bayman, P., Otero J. T. (2010). *Ceratobasidium* como hongo micorrízico de orquídeas en Colombia. *Acta Agronómica*, 59(3), 316–326.

Obour, A. K., Stahlman, P. W., Holman, J. D. (2016). Soil chemical properties as influenced by long-term glyphosate-resistant corn and soybean production in the central Great Plains, USA. *Geoderma,* 277, 1–9. DOI: 10.1016/j.geoderma.2016.04.029.

Otero J.T, Ackerman J.D, Bayman P. (2002). Diversity and Host Specificity of Endophytic *Rhizoctonia*-like Fungi from Tropical Orchids. *American Journal of Botany*, 89(11), 1852–1858.

Otero, J. T., Ackerman, J. D., Bayman, P. (2004) Differences in mycorrhizal preferences between two tropical orchids. *Molecular Ecology*, 13(8), 2393–2404. DOI: 10.1111/j.1365-294X.2004.02223.x

Otero, J. P., Bayman, P., Ackerman, J. D. (2005). Variation in mycorrhizal performance in the epiphytic orchid *Tolumnia variegata* in vitro: the potential for natural selection. *Evolutionary Ecology*, 19(1), 29–43. DOI: 10.1007/s10682-004-5441-0

Otero, J. P., Flanagan, N. S., Herre, E. A., Ackerman, J. D., Bayman, P. (2007). Widespread mycorrhizal specificity correlates to mycorrhizal function in the neotropical, epiphytic orchid Ionopsis utricularioides (Orchidaceae). *American Journal of Botany*, 94(12), 1944–1950. DOI: 10.3732/ajb.94.12.1944

Otero, J.T., Bayman, P. (2009). Germinación simbiótica y asimbiótica en semillas de orquídeas epifitas. *Acta Agronómica*, 58(2), 270–276.

Panwar, D., Ram, K., Harish, Shekhawat N. S. (2012). *In vitro* propagation of *Eulophia nuda* Lindl., an endangered orchid. *Scientia Horticulture,* 139, 46–52. DOI: 10.1016/j.scienta.2012.01.011

Pereira, G., Romero, C., Suz, L. M., Atala, C. (2014). Essential mycorrhizal partners of the endemic Chilean orchids *Chloraea collicensis* and *C. gavilu*. *Flora – Morphology, Distribution, Functional Ecology of Plants*, 209,. 95–99. DOI: 10.1016/j.flora.2013.12.001

Pillon, Y., Fay, M. F., Shipunov, A. B., Chase, M. W. (2006). Species diversity versus phylogenetic diversity: A practical study in the taxonomically difficult genus *Dactylorhiza* (Orchidaceae). *Biological Conservation*, 129, 4–13. DOI: 10.1016/j.biocon.2005.06.036

Puerto, K., Quiroz, A., Talavera, C., Santamaría, J. (2001). Análisis Histológico de Hojas de Plantas de *Cocos nucifera* L. Cultivadas *in vitro*. En A. Aguilar, IX Congreso Nacional de Biotecnología y Bioingeniería, XIII Congreso Nacional de Ingeniería Bioquímica y II Congreso Internacional de Ingeniería Bioquímica. Sociedad Mexicana de Biotecnología y Bioingeniería, Veracruz-México.

Rankin, B. L., Ballantyne, M., Pickering, C. M. (2015). Tourism and recreation listed as a threat for a wide diversity of vascular plants: A continental scale review. *Journal of Environmental Management*, 154, 293–298. DOI: 10.1016/j.jenvman.2014.10.035

Reina-Rodríguez, G. A. & Otero, J. T. (2011). Guía ilustrada de las orquídeas del Valle Geográfico del río Cauca y Piedemonte Andino.

Reina-Rodríguez, G. A., Ospina-Calderón, N. H., Castaño, A., Soriano, I., Otero, J. T. (2010). Catálogo de las orquídeas del Valle Geográfico del río Cauca y su piedemonte Andino Bajo. Sur - occidente colombiano. *CESOEDESIA*, 32(90), 91.

Romero, G. A. Ferreira, I. (2006). Index of botanical specimens. En: Harvard University herbaria & libraries, http://kiki.huh.harvard.edu/databases/specimen_search.php?mode=details&id[]=155268; consulta: noviembre 2015.

Steinmann, H. H., Dickeduisberg, M., Theuvsen, L. (2012). Uses and benefits of glyphosate in German arable farming. *Crop Protection*, 42, 164–169. DOI: 10.1016/j.cropro.2012.06.015

Su, Y. L., Dela Cruz, A., Moore, P. H., Maretzki, A. (1992). The Relationship of Glyphosate Treatment to Sugar Metabolism in Sugarcane: New Physiological Insights. *Journal of Plant Physiology*, 140, 168-173. DOI: 10.1016/S0176-1617(11)80929-6

Tan, M. X., Wang, C. L., Chen X. M.., Zhou, Y.Q., Wang, Y. Q., Luo, A. U., Liu, Z. H., Guo, S. X. (2014). In vitro seed germination and seedling growth of an endangered epiphytic orchid, *Dendrobium officinale*, endemic to China using mycorrhizal fungi (*Tulasnella* sp.). *Scientia Horticulturae*, 165, 62–68. DOI: 10.1016/j.scienta.2013.10.031

Valadares, R. B. D. S., Otero, J. T., Pereira, M. C., & Cardoso, E. J. B. N. (2015). The epiphytic orchids *Ionopsis* *utricularioides* and *Psygmorchis pusilla* associate with different *Ceratobasidium* lineages at Valle del Cauca, Colombia. *Acta Botanica Brasilica*, 29(1), 40–44.

Zhang, Y., Lee, Y., Deng, L., Zhao, S. (2013). Asymbiotic germination of immature seeds and the seedling development of *Cypripedium macranthos* Sw., an endangered lady's slipper orchid. *Scientia Horticulturae*, 164, 130–136. DOI: 10.1016/j.scienta.2013.08.006

Zhang, Z., Yan, Y., Tian, Y., Li, J., He, J., Tang, Z. (2015). Distribution and conservation of orchid species richness in China. *Biological Conservation*, 181, 64–72. DOI: 10.1016/j.biocon.2014.10.026.

Zhao, F., Guo, Y., Huang, Y., Reddy, K., Lee, M. A., Fletcher, R. S., Thomson, S. J. (2014). Early detection of crop injury from herbicide glyphosate by leaf biochemical parameter inversion. *International Journal of Applied Earth Observation and Geoinformation*, 31, 78–85. DOI: 10.1016/j.jag.2014.03.010

LANKESTERIANA 16(2): 279–291. 2016.   doi: http://dx.doi.org/10.15517/lank.v16i2.26012

WHY WE HAVE NO SERIOUS ALTERNATIVES BUT COOPERATIVE TAXONOMY

FRANCO PUPULIN

Lankester Botanical Garden, University of Costa Rica
Harvard University Herbaria, Cambridge, Massachusetts, U.S.A.
The Marie Selby Botanical Gardens, Sarasota, Florida, U.S.A
franco.pupulin@ucr.ac.cr

ABSTRACT. Taxonomic work has been historically regarded as a two-fold discipline. The first, which is basically aimed at answering the question about the diversity in whatever group under study, includes most of the "biological" questions of the research. Understanding of genetic and morphological variation, structure of populations and life cycles, biogeography and phylogeography, ecological modeling, pollination and other biological components is required to define the relationships among the taxa of the group and eventually to describe their diversity. The second part of the work consists in applying a correct name to all of the organisms as they result from the biological work. This second step is usually interpreted as the documentary component of the research, and in fact it mostly deals with the document sources and the rules of biological nomenclature (such as protologues, types and other historical materials associated with the type collections, etc.). However, the use of nomenclatural sources with little or no consideration for the biological aspects of the concerned organisms can be misleading, and the same concept of "type" can be hardly understood if not framed in a rich biological context. Type specimens are just random, individual samples that must be interpreted in the context of the geographical and biological integrity of any given species, and this requires at least some direct knowledge of the organisms and their biology. When the geographical origin of type specimens lies outside the political boundaries of a given study area, taxonomic research is seriously hampered by the impossibility to visualize and understand them in a biological framework. A specific case from the research intended to complete the treatment of the Orchidaceae for the flora of Costa Rica will exemplify how a cooperative approach based on a shared methodology may be the only way to resolve the taxonomy of complex species.

RESUMEN. El trabajo taxonómico ha sido históricamente considerado como una disciplina doble. Por un lado, su objetivo fundamental es responder a la pregunta sobre la diversidad de cualquier grupo bajo estudio, y esto incluye la mayoría de las preguntas "biológicas" de la investigación. Para entender las relaciones y parentescos entre los taxones de un determinado grupo, y finalmente describir su diversidad, se requiere de la comprensión de la variación genética y morfológica, de la estructura de las poblaciones y sus ciclos de vida, de la bio- y filogeografía, de los modelos ecológicos, así como de la polinización y otros componentes bióticos que interactúan con los taxones en estudio. La segunda parte del trabajo consiste en la aplicación de un nombre correcto a cada uno de los organismos así como resultan identificados a través de al etapa biológica del estudio. Esta segunda etapa se interpreta usualmente como la componente documentaria de la investigación, y de hecho tienen mayormente que ver con las fuentes documentales y las reglas de la nomenclatura biológica (tales como protólogos, tipos y otros materiales históricos asociados con las recolectas-tipo, etc.). Sin embargo, el uso de fuentes de nomenclatura asociados a poca o ninguna consideración de los aspectos biológicos de los organismos en estudio puede ser engañoso y el mismo concepto de "tipo" puede difícilmente entenderse si no está enmarcado en un contexto biológico complejo. Los especímenes tipo no son sino ejemplos individuales escogidos al azar, los cuales deben ser interpretados a la luz de la integridad biológica y geográfica de una especie determinada, y esto requiere de por lo menos de algún tipo de conocimiento directo de los organismos y su biología. Cuando el origen geográfico de los especímenes tipo se encuentra afuera de los límites políticos de una determinada área

Received Received 3 May 2016; accepted for publication 16 June 2016. First published online: 30 August 2016.

de estudio, la investigación taxonómica se encuentra seriamente impedida por la imposibilidad de visualizar y entender los tipos en un marco biológico. Un ejemplo del trabajo finalizado a completar el tratamiento de Orchidaceae para la flora de Costa Rica puede enseñar como solamente un acercamiento cooperativo, basado en una metodología común, puede resolver la taxonomía de especies complejas.

Key words: cooperative research, identification, interpretation of types, taxonomy

Introduction. As the branch of science specifically concerned with the description, identification, naming, and classification of organisms, taxonomy has been historically regarded as a two-fold discipline (de Jong 1982). The Greek etymology of the name of the discipline is, in itself, a symptom of this duality. Although there is no ambiguity about the meaning of the ancient Greek word τάξις, taxis, "arrangement, division", the etymology of the second root of the term taxonomy has been referred to both the Greek -νόμος *-nomos*, "law", and -νομία *-nomia*, "method". Even if subtle, the emphasis in the first case is on the orderly classification of organisms according to their presumed evolutionary relationships, whereas in the latter it stresses the main scope of the science at providing an arrangement according to any pre-determined system (a method of classification) to provide a conceptual framework for discussion, analysis, and information retrieval.

The first part of the taxonomic work is indeed aimed at answering the fundamental question about the diversity in the group under study. This activity is still particularly relevant in exploring diversity in the highly biodiverse regions of the planet, where the inventory of life forms is far from complete and more necessary than ever for effective decision-making about conservation and sustainable use. Recent estimates indicate that only 10–20% of living organisms have been discovered/described/catalogued?, the remaining biodiversity numbering perhaps eight million or more species (Wilson 2003). The existence of a taxonomic impediment to the sound management of biodiversity has been already acknowledged by most governments through the Convention on Biological Diversity and the development of the Global Taxonomy Initiative, aimed at reducing the negative effects of insufficient scientific knowledge of life's diversity (Anonymous 1998a, 1998b, Graham 2005). The core missions of alpha-taxonomy, i.e. detecting, identifying, and describing biodiversity units, and supporting these hypotheses with data remain fundamental to all of biology and must be made widely available to address questions outside of taxonomy (Deans *et al.* 2012), even though this simple truth is rarely taken into account by the agendas of institutions and financing agencies (Flowers 2007). Fundamental questions from other disciplines such as speciation, diversification processes, ecosystem development, and conservation priorities depend on correct species boundaries and diversity estimates (Dayrat 2005, Padial *et al.* 2010). From its beginnings, by using the phenotype, taxonomy has progressed with the continuous use of different tools, evolving enough to enable a better understanding of how genes control morphology today. In order to provide crucial data on ecology, evolution, and biogeography, and a rational framework for phylogenetic and phylogeographic studies, "modern" taxonomy (whatever this frequently used expression means) requires intensive collecting and observation, detailed measurement, description, and illustration, as well as information at the cellular and molecular levels (Bicudo 2004).

It is true that merely collecting and describing new species does not advance our understanding of life (Ehrlich 1961), but it has to be remembered that the process of describing life's diversity is fundamentally aimed at identifying species, not specimens (Dick & Mawatari 2004). As such, taxonomy deals with groups of individuals (specimens) that show, to a greater or lesser degree, intraspecific variability. The understanding of the morphology and evolution of a particular group, which is expressed in polymorphism and natural phenotypic variability, is essential to define the relationships among the taxa of the group and eventually describe their real diversity. It cannot be stressed enough how a reliable taxonomy must be an information-intensive field, which requires not only a firsthand knowledge of a large sample of populations and individuals belonging to the concerned groups, but also information on the key characters driving the evolution of a particular group, the training in interpreting centuries of previous literature, as well as a comparative understanding of the past and present schemes of classification.

Even though the floras of bio-rich tropical countries were mostly made by scientists working and studying outside the natural environments of the concerned taxa, it is today broadly recognized that modern floristics require appreciation of natural variation (Ellison *et al.* 2014), so relying on the fine comparison of broadly sampled specimens cannot be carried out without a local school of trained botanists. Such an approach produces useful classification systems more easily, which allow the delimitation and identification of species in such a way that they exhibit genetic differences and ecological preferences (McNeill 1976). A great familiarity with local geography, the appreciation of the taxonomic implications of fine-tuned ecological mapping, direct knowledge of the historical background of botanical exploration, and ample access to the examination of both living and pressed specimens greatly improve the accuracy and reliability of floristic catalogues, identification of critical materials, description of new taxa, and formulation of hypotheses on evolutionary relationships among living organisms.

For as accurate as it might be, the detection and identification of the diversity of living organisms in a given region represents only the first step along the path aimed to produce a catalogue of life. This catalogue, in fact, requires the correct naming of the retrieved organisms and groups, both at the specific level and at the supra- and infra-specific categories. The diversity of life on Earth can neither be appreciated nor communicated without a semantic system that can name it in a consistent and repeatable way (Patterson *et al.* 2010). Nomenclature is the second essential element of taxonomy. It is governed by a set of rules that date back to the *lois de Candolle* in 1867 (de Candolle 1867), through the “Vienna Rules” of 1906 (Briquet *et al.* 1906) and the “Stockholm Code” of 1952, and has been since continuously updated and adapted to the new needs of science up to the recent Melbourne Code, adopted in 2011 (McNeill *et al.* 2011).

One of the crucial rules of the Code is that the name of a taxon is permanently attached to “a nomenclatural type (*typus*)” (McNeill *et al.* 2011: art. 7). The *typus* is an example that serves to anchor the defining features of that particular species and a reference point when attempting to determine the correct application of a name.

Tropical botany (and orchidology is not an exception) has been traditionally hampered by the difficulty to access type specimens for comparison and application of names, as most of the types were kept in European and North American herbaria and museums. To circumvent the difficulty of accessing, and the delicacy of conserving, historical specimens, images of types in the form of photographs, slides, photocopies, etc. have been used as complementary materials for taxonomic studies, but it was not until the advent of digital data capture in the last few decades that the information sources represented by biological collections kept in developed countries began to be disseminated effectively (Pupulin 2013). Today, the use of the Internet and the increasing availability both of digitized historical literature and images of plant types from all the major herbaria around the world have amplified visual access to the most important sources of nomenclatural concepts. The Global Plants Initiative, an international undertaking by leading herbaria to digitize and make types available, includes more than 270 partners in 70 countries and holds nearly two million high-resolution images of plant type specimens (La Monica 2013). Although such images offer the advantage of their instantaneous accessibility, the examination of the actual specimens is still considered the preferred method for several types of scientific research, because critical features may be difficult to discern from two-dimensional images (Culley 2013). There is, however, an additional argument to be discussed relative to the study and understanding of plant type specimens.

A biological approach to type specimens and species concepts. Type specimens are random individuals from a given population, randomly collected and designated as a reference to fix the combination of key and unique features associated with that particular species concept. As such, types represent only one point within a continuum of genetic and morphological differences among members of the same species, as well as across its entire geographic range, and not necessarily the most representative element of its own taxon. Understanding the extent of natural variation among individuals or populations of a single species is a fundamental task for the botanist. Correct interpretation of nomenclatural types gives the botanist the ability to assign individuals to groups using clear phenotypic distinctions that (may or may not) reveal underlying genetic differences (Mather & Jinks 1982) and ensures that the material which typically corresponds to that species falls within the continuous range of variation provided by their descriptions.

Variation in plant morphology could be, on the other hand, a function of phenotypic responses to selection pressures and thus reflect the evolutionary history of populations. Several morphological features are genetically constrained but also greatly affected by the specific conditions of the environment in which they develop (Ellison *et al.* 2004). In order to understand if morphological variation is associated with environmental and climatic gradients, the expected geographic range of a species must be known, and a broad sampling of individuals is required, including specimens sympatric with the type as well as individuals from disjunct populations. Genetic sampling, including a broad representation of individuals from distinct populations and possibly specimens from the type localities, accompanied by a rigorous record of individual salient features, would also immensely help in assessing univocal species circumscriptions. Genetic characterization should ideally be done locally according to shared protocols, in order to circumvent the growing difficulties in moving samples across political boundaries for molecular analyses.

In any of these scenarios, an exhaustive survey and a precise documentation of individual variation, or phenome annotations, at the type locality are required for the correct interpretation and unambiguous understanding of the type specimen. Next-generation sequencing (NGS) promises to be of major utility for the study of herbarium specimens, including types. Most NGS methods are designed for using as templates short fragmented DNA molecules, and the recovering of entire nuclear genomes from old herbarium specimens has already been carried out with success (Straub *et al.* 2012, Staats *et al.* 2013). Only a rich biological approach, which takes into account complementary perspectives – phytogeography and phylogeography, genetics, pollination ecology, among others –guarantees that the boundaries of a given species can be accurately traced from the study of the type. As plant distribution is irrespective of political boundaries, it is frequent in taxonomic research that the type locality lies outside the study region.

The drawback of the integrative approach is that it can be carried out only at the local level, where populations at the type locality or close to it still exist and may be sampled for critical study. The advantage of the method, however, is that it requires training and involvement in the research for local students, who must possess the phytogeographic, ecological, and taxonomic skills required to document intra- and interspecific variation and compare it with the set of individual features that are specific to the type specimens.

A closer look at a specific case may clearly exemplify the need of a cooperative approach to the interpretation of the types and to a sound application of names.

The enigmatic tailed *Brassia*. *Brassia caudata* (L.) Lindl. has been treated as the most widespread species of the genus, including records from the whole of tropical America. It has been recorded from Florida, Jamaica, Cuba, the Dominican Republic and Haiti, Trinidad, Mexico, Guatemala, Belize, El Salvador, Nicaragua, Costa Rica, Panama, Venezuela, Surinam, Guyana, Brazil, Colombia, Ecuador, Peru, and Bolivia (Cogniaux 1906, Fawcett & Rendle 1910, Williams & Allen 1946, Ames & Correll 1953, Foster 1958, Schultes 1960, Schweinfurth 1961, Dunsterville & Garay 1966, Foldats 1970, Long & Lakela 1971, Hamer 1974, Dressler 1980, Hamer 1982, Vásquez & Dodson 1982, Breedlove 1986, Werkhoven 1986, Dodson & Bennett 1989, Atwood & Mora de Retana 1993, Brako & Zarucchi 1993, Dodson 1993, Dressler 1993, Bennett & Christenson 1995, Gloudon & Tobisch 1995, McLeish *et al.* 1995, Steyermark *et al.* 1995, Wunderlin 1998, Jørgensen & León-Yánez 1999, Balick *et al.* 2000, Nir 2000, Carnevali *et al.* 2001, Martínez *et al.* 2001, Stevens *et al.* 2001, Fernández 2003, Dodson & Luer 2005, Llamacho & Larramendi 2005, Pupulin 2005, Misas Urreta 2006, Fernández 2007, Ospina H. 2008, Zelenko & Bermúdez 2009, Luz & Franco 2012, Rakosy *et al.* 2013). Morphologically, these records are highly variable, sharing basically the caudate lateral sepals, a common and probably plesiomorphic feature in the genus (Fig. 1). In Costa Rica, at least two taxonomic entities broadly distinct on the ground of geographic distribution, morphological features, and fragrance (Fig. 2–3), have been treated as members

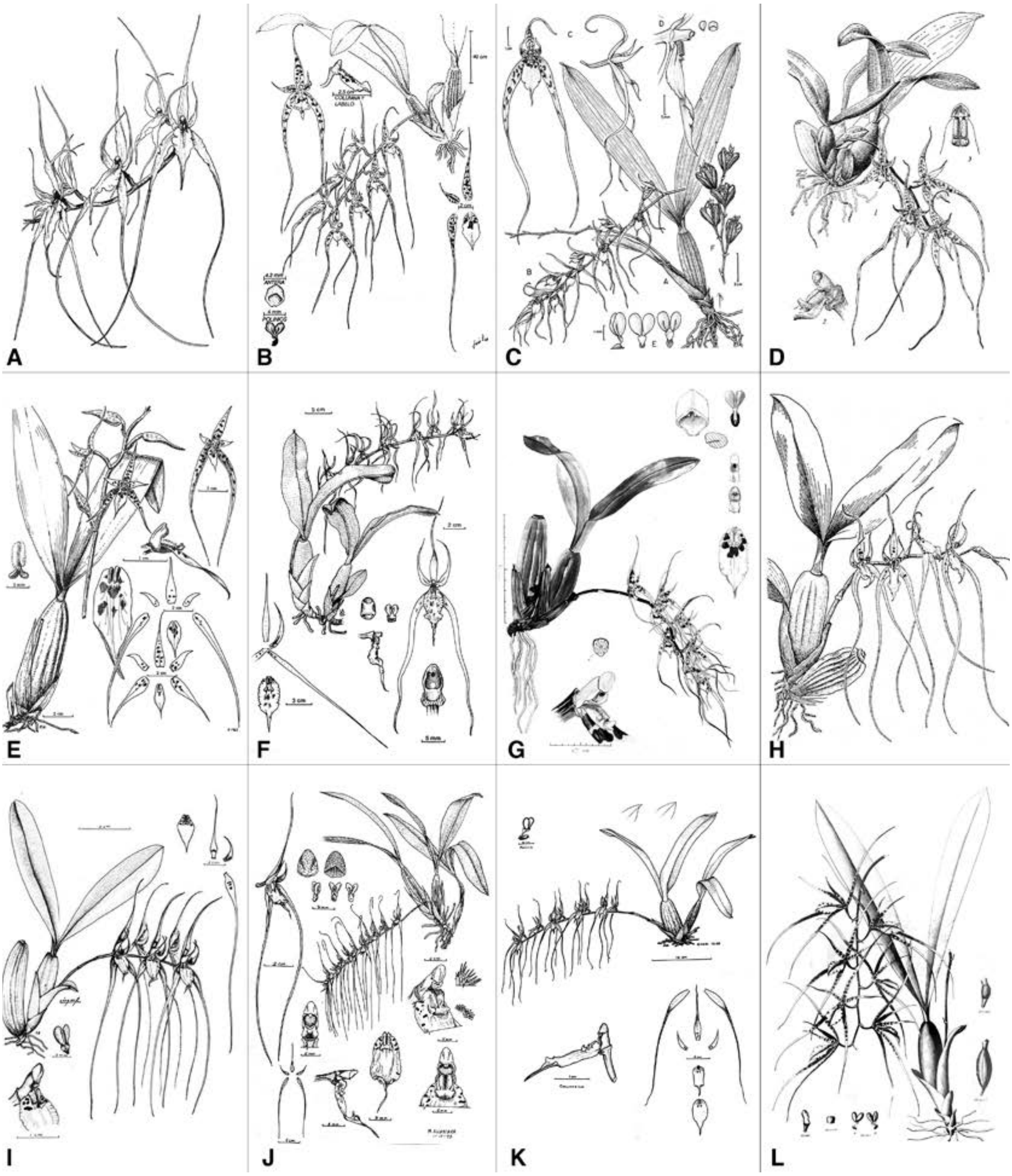


FIGURE 1. Illustrations from specimens identified as *Brassia caudata* from different regions. A, Florida (from Ames 1947). B, Cuba (from Mújica Benítez *et al.* 2000). C, Cuba and Jamaica (composite plate from Ackerman 2015). D, Guatemala (from Ames & Correll 1953). E, Nicaragua (from Hamer 1982). F–G, Costa Rica (from Atwood & Mora 1993, and Rodríguez Caballero *et al.* 1986, respectively). H, Panama (from Williams & Allen 1946). I, Colombia (from Escobar R., 1990). J, Peru (from Bennett & Christenson 1995). K, Venezuela (from Dunsterville & Garay 1966). L, Brazil (From Cogniaux 1906).

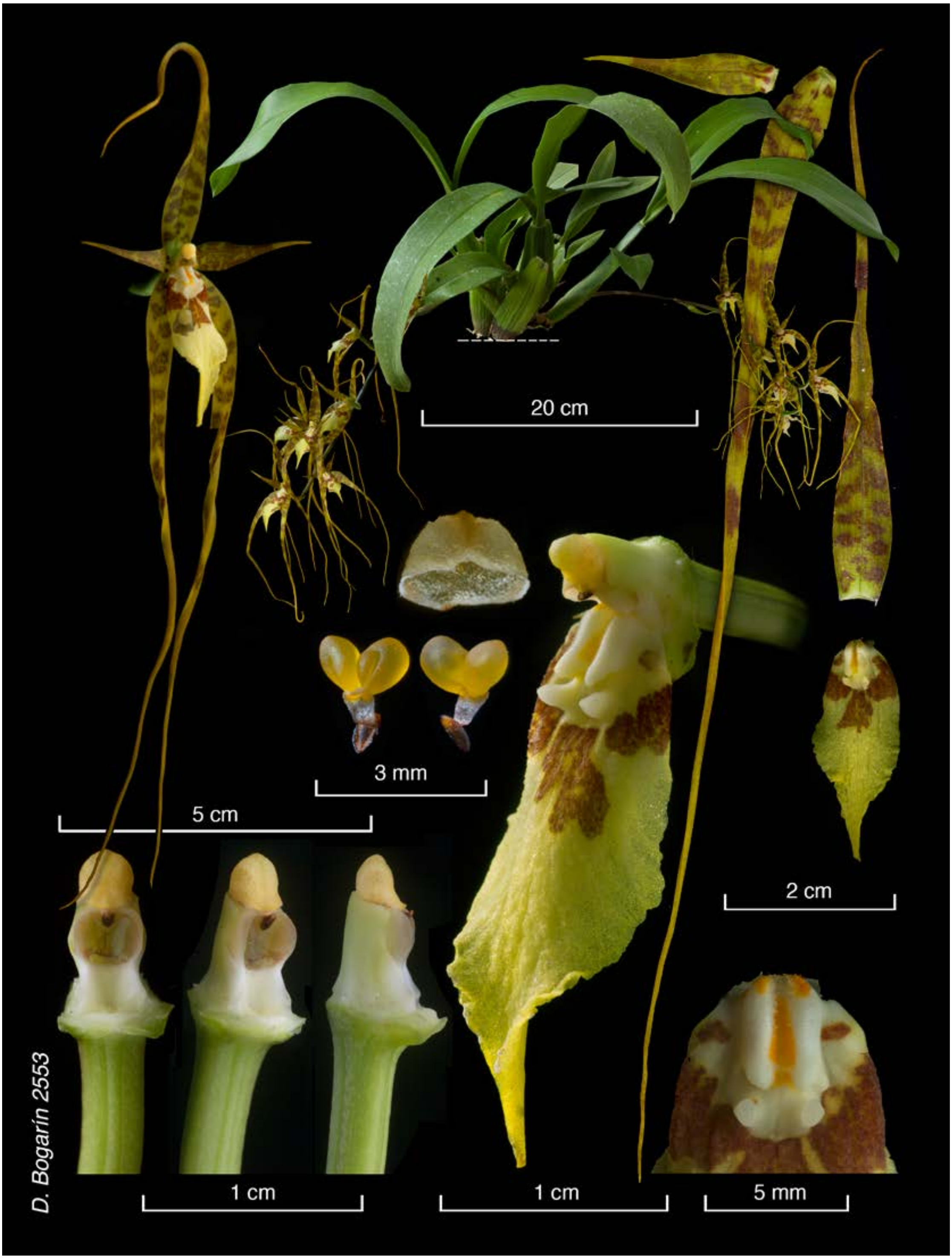


Figure 2. Lankester Composite Digital Plate of *Brassia* cf. *caudata*. Costa Rica, Caribbean morph, *D. Bogarín 2553* (voucher: JBL).

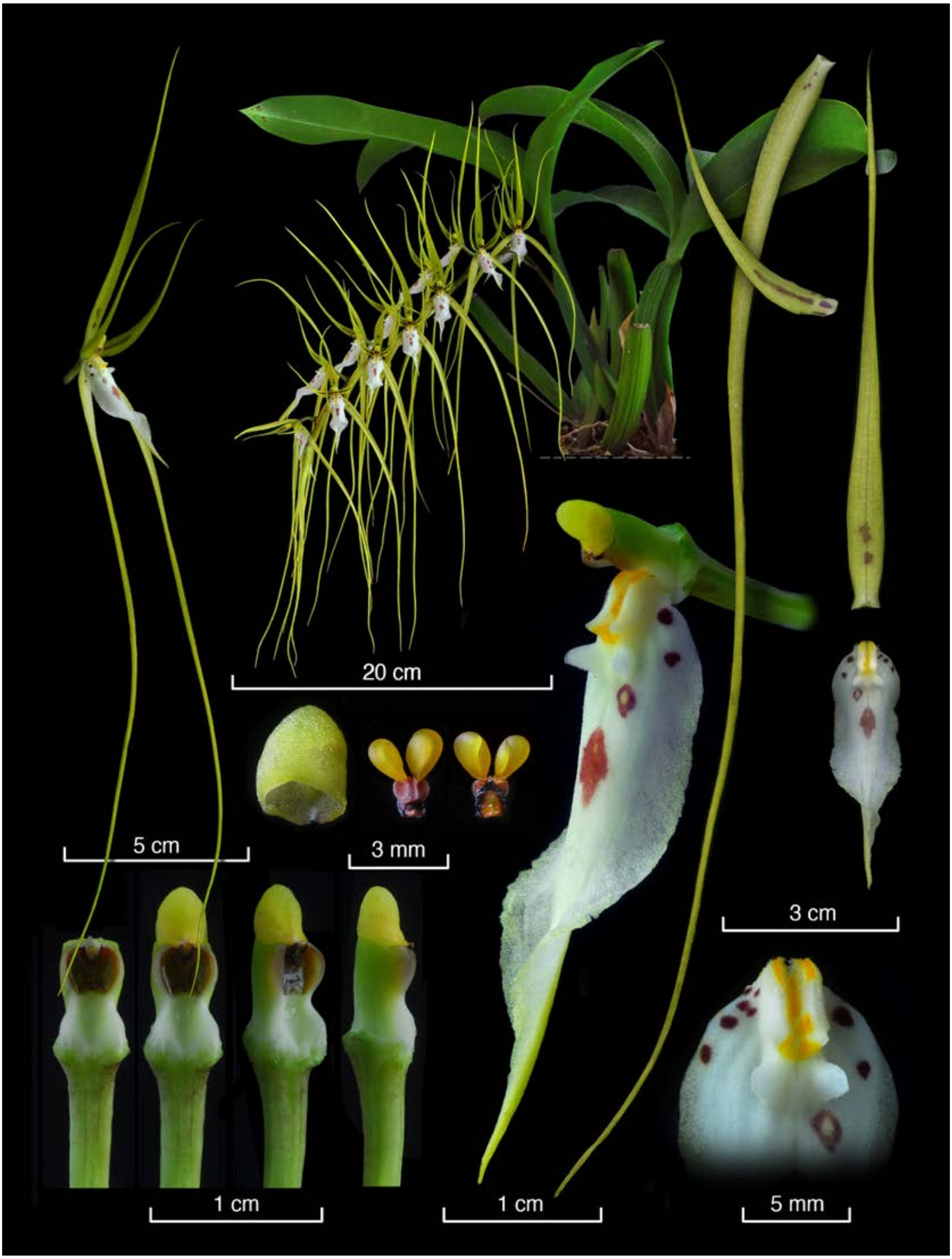


Figure 3. Lankester Composite Digital Plate of *Brassia* cf. *caudata*. Costa Rica, Pacific morph, *JBL-21704* (voucher: JBL).

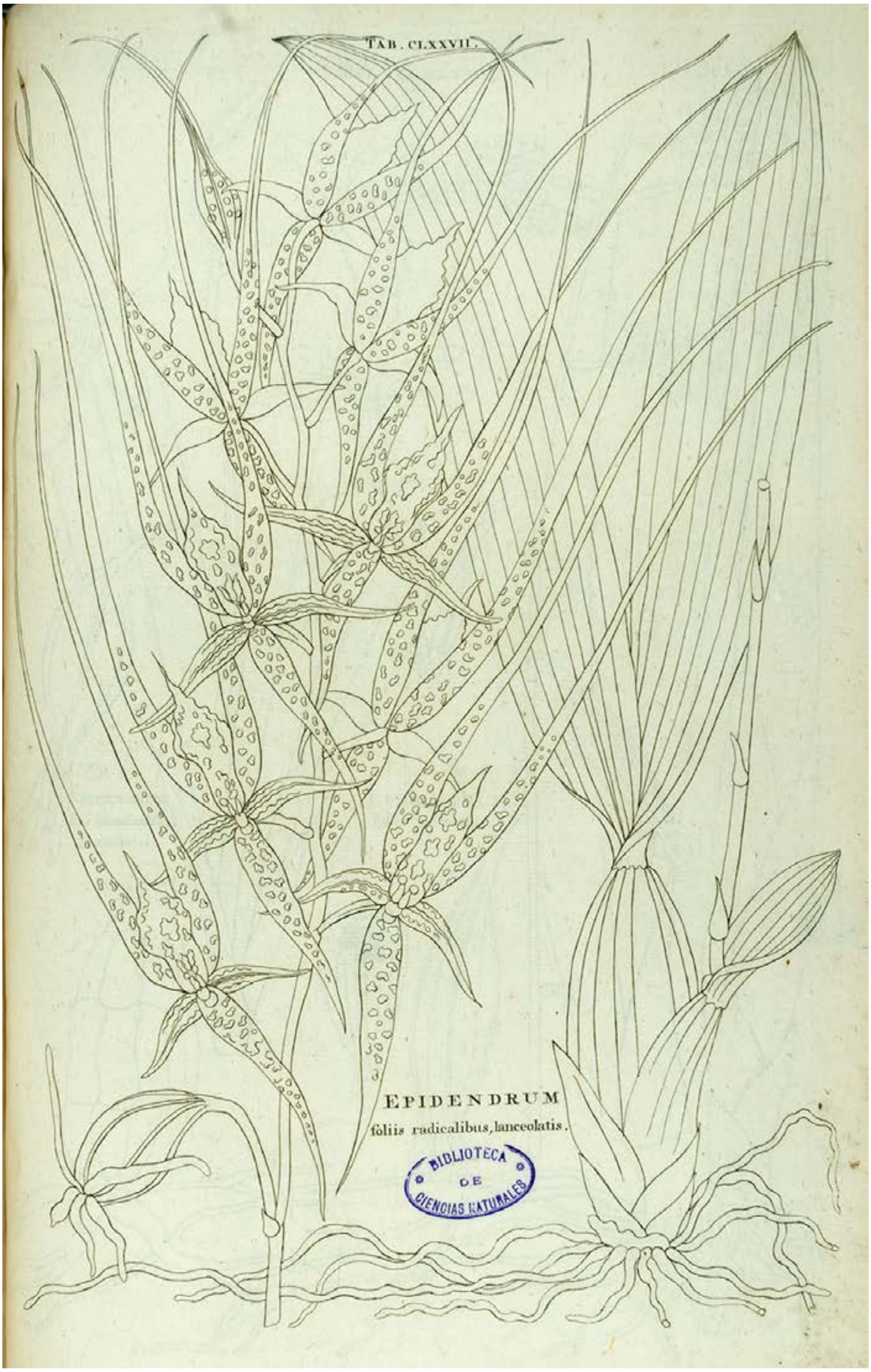


Figure 4. Johannes Burman's illustration of Plumier's "Epidendrum foliis radicalibus lanceolatis" (Plumier 1758), which McLeish and collaborators (1995) selected as the type for the species.

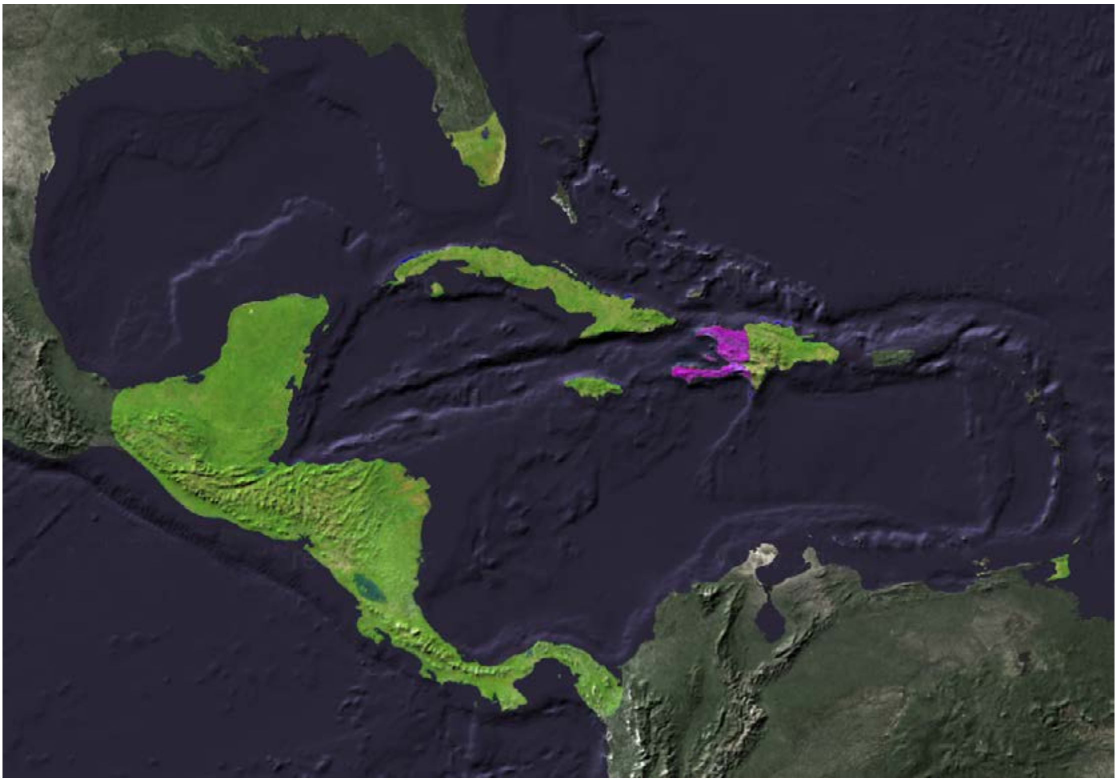


FIGURE 5. Distribution map of *Brassia* cf. *caudata* populations in Central America and the Caribbean (light green). Haiti, where Plumier botanized during his trip, is indicated in magenta.

of this poorly defined species (Atwood & Mora 1993, Pupulin & Bogarín 2005), and at least one of the Costa Rican taxa has been treated as the Panamanian record of *B. caudata* (Williams & Allen 1946).

The name *Brassia caudata* is based on Linné's (1758-1759) *Epidendrum caudatum*, the type of which is an illustration by Johannes Burman (in Plumier 1758) (Fig. 4) from a plant traditionally treated as collected "in the West Indies." During his American trips, Plumier mostly worked in the West Indies' French dominions – Haiti, Martinique and Guadaloupe – but also visited the Virgin Islands and some other islands of the Lesser Antilles, including St. Christopher, St. Croix, and the Grenadines of St. Vincent. Ackerman (2015) has recently reconsidered the actual distribution of *Brassia* species with caudate sepals in the West Indies and recorded it exclusively for the islands of Cuba, Jamaica, and Hispaniola (both in Haiti and the Dominican Republic) in the Greater Antilles, and the island of Trinidad in the Lesser Antilles (Fig. 5). If Burman's illustration of "Epidendrum foliis radicalibus lanceolatis" was based on a collection made by Plumier, the evidence indicates that the original specimen must have been collected in the territory of present-day Haiti. At Lankester Botanical Garden we do not have any plant of *Brassia* native to the island of Hispaniola, but we do grow a specimen from Jamaica, which Claude Hamilton gave to Bob Dressler many years ago (Fig. 6). The features of this plant raise the suspicion that Jamaican populations referred to *B. caudata* could perhaps represent a different taxon when compared to the specimen illustrated by Plumier in his "*Tabula centesima septuagesima septima*", or may indicate instead that more than one species of *Brassia* with tailed sepals inhabits the island. The only evidence available at Lankester Botanical Garden of a *B. caudata* specimen from the Greater Antilles also raises legitimate doubt about the identity of both taxa traditionally treated as *B. caudata* in Costa Rica, the correct taxonomy of other regional populations in the Central American isthmus, and the taxonomic position of the South American species included in a broad and ill-defined concept of *B. caudata*.

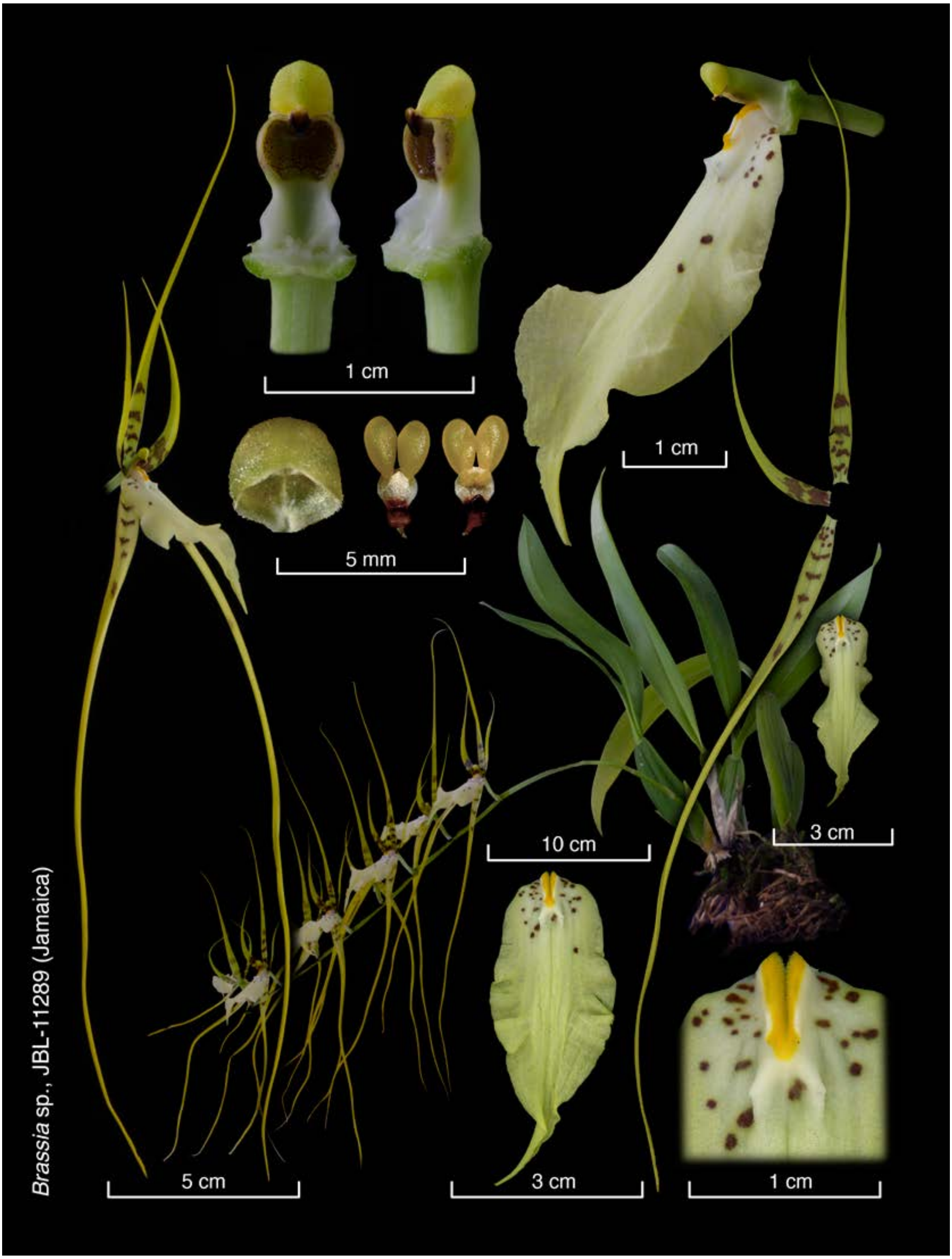


Figure 2. Lankester Composite Digital Plate of *Brassia* cf. *caudata*. Costa Rica, Caribbean morph, *D. Bogarín 2553* (voucher: JBL).

These uncertainties cannot be resolved internally in Costa Rica, nor in other continental countries in America, as the basic questions relative to the taxonomic identity of the true *B. caudata* have to be answered in the West Indies! Can a true *B. caudata* from Haiti, documented with modern imaging techniques, stand up? Can the natural variation of this species be documented and estimated at the type locality? Can the true *B. caudata* be compared, using similar techniques, with populations recorded from the Dominican Republic, Cuba and Jamaica? Is there a possible answer to the question about the diversity of *Brassia* in Jamaica and the Greater Antilles, spanning the greatly discordant morphologies of the plant collected by Plumier and the one from Jamaica grown at Lankester Botanical Garden?

It is clear that the identification of the caudate *Brassia* populations occurring from Mexico to Brazil and Bolivia requires a modern and integrative approach to the interpretation of the type that subtends the concept of *B. caudata*. It is also easy to foresee how this approach must be supported by the cooperative effort by a group of local botanists who share similar research methodologies, aimed at clarifying the status of local populations and taxa, with a preliminary emphasis on the understanding of the characteristics and range of morphological and genetic variation recorded at the type locality.

Conclusions. Just as integrative taxonomy was defined as the science aimed at delimiting the units of life's diversity from multiple perspectives (Dayrat 2005), so the integrative interpretation and the biological approach to type specimens, which incorporates complementary disciplines, are fundamentally aimed at furthering our understanding of the species concept. This integration not only allows a critical revision of the previously described species' classification, but also enhances the capacity of detecting, describing, and classifying new species in a rich biological context. The basis of the integrative study of types is not the work in the herbarium, but a complex of rigorous phenome observations that produce information of taxonomic and evolutionary value and provide a conceptual framework for the actual study of type specimens.

This methodology necessarily requires a strict local approach, which has to be mainly carried out at the type localities, as only a sharp understanding of nomenclatural types allows for a stable recognition of new phenotypes as the same as, or different from, the types of previously known taxa. After centuries of colonial botany, which in several cases had as a result a poor understanding of species boundaries and a limited appreciation of the taxonomic value of type specimens, the integrative study of types represents a powerful stimulus for cooperative research and the sharing of common methodologies.

ACKNOWLEDGMENTS. I thank my colleagues at the Lankester Botanical Garden, with whom I shared most of the concepts expressed here, and warmly acknowledge their patience in listening again and again to the topics that I summarized in this article while they gradually took shape in our discussions. A particular acknowledgment is due to Robert Dressler, who first introduced me to the idea of a biological interpretation of type specimens. The suggestions and corrections by anonymous reviewers greatly improved the manuscript.

LITERATURE CITED

Ackerman J.D. (2015). Orchid flora of the Greater Antilles. Mem. New York Bot. Gard., 109, 1–625.

Ames B. (1947). Drawing of Florida orchids. With explanatory notes by Oakes Ames. Botanical Museum, Cambridge, Mass.

Ames O. & Correll D.S. (1953). Orchids of Guatemala. Fieldiana, Botany, 26(2), 399–727.

Anonymous (1998a). The Global Taxonomy Initiative: shortening the distance between discovery and delivery. Environment Australia, Canberra.

Anonymous (1998b). The Global Taxonomy Initiative: using systematic inventories to meet country and regional needs. Diversitas, Systematics Agenda 2000. American Museum of Natural History, New York.

Atwood J.T. & Mora de Retana D.E. (1999) Family #39 Orchidaceae: Tribe Maxillarieae: subtribes Maxillariinae and Oncidiinae. Fieldiana, Botany, New Series, 40, i-vi, 1–182.

Balick M.J., Nee M.H. & Atha D. E. (2000). Checklist of the vascular plants of Belize. Mem. New York Bot. Gard., 85, i–ix, 1–246.

Bennett D.E. Jr. & Christenson E.A. (1995). Icones Orchidacearum Peruviarum, Volume 3. Privately published by A. Pastorelli de Bennett, Lima.

Bicudo C.E. de M. (2004). Taxonomia. Biota Neotrop., 4(1), i-ii.

Brako L. & Zarucchi J.L. (1993). Catalogue of the Flowering Plants and Gymnosperms of Peru. Monog. Syst. Bot. Missouri Bot. Gard., 45, i–xl, 1–1286.

Breedlove D.E. (1986). Flora de Chiapas. Listados Florísticos de México, 4, i-v, 1-246.

Briquet J., Flahault C., Harms H. & Rendle A.B. (1906). International Rules of Botanical Nomenclature, adopted by the International Botanical Congress of Vienna, 1905. G. Fischer, Jena.

Carnevali G., J.L. Tapia-Muñoz J.L., R. Jiménez-Machorro R., L. Sánchez-Saldaña L., L. Ibarra-González L., I.M. Ramírez I.M. & M.P. Gómez M.P. (2001). Notes on the flora of the Yucatan Peninsula II: a synopsis of the orchid flora of the Mexican Yucatan Peninsula and a tentative checklist of the Orchidaceae of the Yucatan Peninsula biotic province. Harvard Pap. Bot., 5(2), 383-466.

Cogniaux, A. (1906) Orchidaceae. *In*: C.F.P. von Martius (ed.). Flora Brasiliensis, 3 (6). Fleischer, Leipzig.

Culley T.M. (2013). Why vouchers matter in botanical research. Appl Plant Sci., 1(11), doi: 10.3732/apps.1300076.

Dayrat B. (2005). Integrative taxonomy. Biol. J. Linnean Soc., 85, 407–415.

de Candolle M.A. (1867). Lois de la nomenclature botanique adoptées par le Congrès international de botanique tenu à Paris en août 1867… H. Georg, Geneva and Basel.

Dick M.H. & Mawatari S.F. (2004). Resolving taxonomic problems of North Pacific bryozoans. Pp. 67–74 in: S.F. Mawatari & H. Okada (eds.), Neo-science of natural history: integration of geoscience and biodiversity Studies. Proceedings of International Symposium on "Dawn of a New Natural History - Integration of Geoscience and Biodiversity Studies", March 5-6, 2004, Sapporo.

Deans A.R., Matthew J.Y. & Balhoff J.P. (2012). Time to change how we describe biodiversity. Trends Ecol. Evol., 27(2), 78–84.

de Jong, R. (1982). The biological species concept and the aims of taxonomy. J. Res. Lepidopt., 21(4), 226–237.

Dodson C.H. (1993). Native Ecuadorian Orchids. 3. Dodson Trust, Sarasota.

Dodson C.H. & Bennett D.E. Jr. (1989). Orchids of Peru, Icones Plantarum Tropicarum, Series 2, Fasc. 1–2, 1–200.

Dodson C.H. & Luer C. (2005). Orchidaceae. Genera *Aa-Cyrtidiorchis*. *In*: G. Harling & L. Andersson (eds.). Flora of Ecuador 76, 1–345. University of Götenborg, Riksmuseum.

Dressler R.L. (1980). Checklist of the orchids of Panama. Pp. i-xxvi *in*: Orchids of Panama: A facsimile reprint of the Orchidaceae, flora of Panama. Missouri Botanical Garden Press, St. Louis.

Dressler R.L. (1993). Field guide to the orchids of Costa Rica and Panama. Cornell University Press, Ithaca.

Dunsterville G.C.K. & Garay L.A. (1966). Venezuelan Orchids Illustrated: Volume 4. Andre Deutsch, London.

Ehrlich P.R. (1961). Systematics in 1970: some unpopular predictions. Syst. Zool., 10, 157-158.

Ellison A.M, Buckley H.L., Miller T.E. & Gotelli N.H. (2004). Morphological variation in *Sarracenia purpurea* (Sarraceniaceae): geographic, environmental, and taxonomic correlates. Amer. J. Bot., 91(11), 1930–1935.

Ellison A.M, Davis C.C., Calie P.J. & Naczi R.F.C. (2014). Pitcher plants (*Sarracenia*) provide a 21st-century perspective on infraspecific ranks and interspecific hybrids: a modest proposal for appropriate recognition and usage. Syst. Bot., 39(3), 939–949.

Escobar R.R. (ed.). (1990). Orquideas nativas de Colombia. Vol. 1: *Acacallis-Dryadella*. Compañía Litográfica Nacional, Medellín.

Fawcett W. & Rendle A.B. (1910). Flora of Jamaica, containing descriptions of the flowering plants known from the island. The British Museum, London.

Fernández C. (2003). Orquídeas Orquídeas nativas del Táchira. Editorial Litolifa, San Cristobal, Táchira.

Fernández E. (2007). Orquídeas de la República Dominicana y Haití / Orquídeas of the Dominican Republic and Haiti. American Chamber of Commerce of the Dominican Republic, Santo Domingo.

Flowers R.W. (2007). Comments on "Helping Solve the 'Other' Taxonomic Impediment: Completing the Eight Steps to Total Enlightenment and Taxonomic Nirvana" by Evenhuis (2007). Zootaxa, 1494, 67–68.

Foldats E. (1970). Orchidaceae. *In*: T. Lasser, Flora de Venezuela 15(1): 169-201. Instituto Botánico, Dirección de Recursos Renovables, Ministerio de Agricultura y Cría, Caracas.

Foster R.C. (1958). A catalogue of the ferns and flowering plants of Bolivia. Contr. Gray Herb. Harvard Univ., 184, 1–223.

Gloudon A. & Tobisch C. (1995). Orchids of Jamaica. University of West Indies Press, Jamaica.

Graham M. (2005). The Global Taxonomic Initiative. Canad. Bot. Assoc. Bull., 38(3), 35–36.

Hamer F. (1974) Las Orquideas de El Salvador I. Ministerio de Educacion, San Salvador.

Hamer, F. (1982). Orchids of Nicaragua. Part 2. Icon. Pl. Trop., Fasc. 8, 701–800.

Jørgensen P.M. & León-Yánez S. (eds.). (1999). Catalogue of the vascular plants of Ecuador. Monogr. Syst. Bot. Missouri Bot. Gard., 75, i–viii, 1–1182.

La Monica M. (2013). The Global Plants Initiative and JSTOR release online database for the study of plants. At: http://about.jstor.org/news, retrieved January 2016.

Llamacho J.A. & Larramendi J.A. (2005). The orchids of Cuba / Las orquídeas de Cuba. Greta Editores, Lleida, España.

Linné C. von (1758-1759). Systema naturae per regna tria naturae… (ed. 10). Impensis Direct. Laurentii Salvii, Stockholm.

Long R.W. & Lakela O. (1971). A Flora of Tropical Florida. University of Miami Press, Coral Gables.

Luz J. & Franco J. (2012). Orquídeas de Roraima. Empresa Brasiliera de Pesquisa Agropecuária, Embrapa Roraima, Ministério da Agricultura, Brasília, DF.

Martínez E., Sousa S.M. & Ramos Álvarez C. H. (2001). Región de Calakmul, Campeche. Listados Florísticos de México, 22, 1-55.

Mather K. & Jinks J.L. (1982). Biometrical genetics: the study of continuous variation. Third ed. Chapman and Hall, London.

McLeish I., Pearce N.R. & Adams B. R. (1995). Native Orchids of Belize, 1-278. Balkema, Rotterdam.

McNeill J. (1976). The taxonomy and evolution of weeds. Weed Res., 16(6), 399–413.

McNeill J., Barrie F.R., Buck W.R., Demoulin V., Greuter W., Hawksworth D.L., Herendeen P.S., Knapp S., Marhold K., Prado J., Prud'homme van Reine W.F., Smith G.F., Wiersema J.H. & Turland N.J. (2011). International Code of Nomenclature for algae, fungi, and plants (Melbourne Code), adopted by the Eighteenth International Botanical Congress, Melbourne, Australia, July 2011. Regnum Veg., 154 [Appendices II–VIII in Regnum Veg. 157].

Misas Urreta, G. (2006). Orquídeas de la Serranía del Baudó, Chocó, Colombia / Orchids from the Serranía del Baudó, Chocó, Colombia. Corporación Capitalina de Orquideología, Bogotá.

Mújica Benítez E., Pérez Márquez R., Bocourt Vigil J.L., López Trabanco P.J. & Ramos Calderón T.M. (2000). Géneros de orquídeas cubanas. Editorial Félix Varela, La Habana.

Nir, M. A. (2000). Orchidaceae Antillanae. DAG Media Publishing, Inc., New York.

Ospina H.M. (2008). Las orquídeas Oncidiineas de Colombia / The Colombian Oncidiinae orchids. Ediciones Ántropos, Bogotá.

Padial J.M., Miralles A., De la Riva I. & Vences M. (2010) The integrative future of taxonomy. Front. Zool., 7, 1–16,

Patterson, D.J., Cooper J., Kirk P.M., Pyle R.L. & Remsen D.P. (2010). Names are key to the big new biology. Trends Ecol. Evol. 25, 686–691.

Plumier C. (1758). Plantarum americanarum fasciculi decem, continentes plantas, quas olim… Sumtibus Auctoris, Prostant Amstelædami [Amsterdam] in Horto Medico, Atque apud Petrum Schouten, & apud Gerard. Potvliet, & Theodor. Haak., Leiden.

Pupulin F. (2005). *Vanishing Beauty. Vol. 1*: *Acianthera-Kegeliella*. Editorial de la Universidad de Costa Rica, San José.

Pupulin F. (2013). Toward a global orchid taxonomic network. Lankesteriana, 13(1–2), 75–91.

Pupulin F. & Bogarín D. (2005). The genus *Brassia* in Costa Rica: a survey of four species and a new species. Lindleyana in Orchids (Bull. Amer. Orch. Soc.), 74(3), 202—207.

Rakosy D., Speckmaier M., Weber A., Huber W. & Weissenhofer A. (eds.). (2013). Orchids. Botanical jewels of the Golfo Dulce Region, Costa Rica. Verein zur Förderung der Tropenstation La Gamba, Vienna.

Rodríguez Caballero R.L., Mora D.E., Barahona M.E. & Williams N.H. (1986). Géneros de Orquídeas de Costa Rica. Editorial Universidad de Costa Rica, San José.

Schweinfurth C. (1961). Orchids of Peru. Fieldiana, Botany, 30(4), 787-1005.

Schultes R.E. (1960). Native Orchids of Trinidad and Tobago. Pergamon Press, New York.

Staats M., Erkens R.H.J. van de Vossenberg B., Wieringa J.J., Kraaijeveld K., Stielow B., Geml J., Richardson J.E. & Bakker F.T. (2013). Genomic treasure troves: complete genome sequencing of herbarium and insect museum specimens. PlosOne 8(7): e69189. doi:10.1371/journal.pone.0069189

Stevens W.D., Ulloa U.C., Pool A. & Montiel O.M. (2001). Flora de Nicaragua. Monogr. Syst. Bot. Missouri Bot. Gard., 85, i–xlii, 1–2666.

Steyermark J.A., Berry P.E. & Holst B.K. (eds). Flora of Venezuelan Guayana, vol. 7. Missouri Botanical Garden Press and Timber Press, St. Louis and Portland.

Straub S.C.K., Parks M., Weitemeir K., Fishbein M., Cronn R. & Lston A. (2012). Navigating the tip of the genomic iceberg: next-generation sequencing for plant systematics. Am. J. Bot. 99: 349–364.

Vásquez R. & Dodson C. H. (1982). Orchids of Bolivia. Icones Plantarum Tropicarum, Series 1, Fasc. 6, 501–600.

Williams L.O. & Allen P. H. (1946). Orchidaceae. *In*: R.E. Woodson, Jr. & R.W. Schery. Flora of Panama. Part III, Fascicle 4. Ann. Missouri Bot. Gard., 36.

Werkhoven M.C.M. (1986). Orchideeen van Suriname / Orchids of Suriname. Vaco, Uitgeversmaatschappij, Paramaribo.

Wilson E. (2003). The encyclopedia of life. Trends Ecol. Evol., 18, 77–80.

Wunderlin R.P. (1998). Guide to the vascular plants of Florida. University Presses of Florida, Tampa.

Zelenko H. & Bermúdez P. (2009). Orchids Species of Peru. Zai Publications, Quito.

LANKESTERIANA 16(2): 293–297. 2016.   **doi**: http://dx.doi.org/10.15517/lank.v16i2.26013

DETERMINANTS OF ORCHID SPECIES DIVERSITY IN LATIN AMERICA

Zuzana Štípková[1,2], Iva Traxmandlová[1] & Pavel Kindlmann[1–3]

[1] Global Change Research Institute, Academy of Sciences of the Czech Republic, Prokišova 356/7, České Budějovice, 37001, Czech Republic

[2] Institute for Environmental Studies, Faculty of Science, Charles University, Benátská 2, Prague, 12801, Czech Republic

[3] Author for correspondence: pavel.kindlmann@centrum.cz

Abstract. Area and latitude are thought to be the most important determinants of species richness. The relative importance of these two factors was recently tested, using data on orchid species diversity in various countries in the world and it was found that size of the country (or of the protected areas within the country) is a better determinant of species diversity in orchids than latitude. On the other hand, literature data indicate that in many groups species richness is also heavily dependent on habitat diversity as expressed by the range of altitudes in the region considered. Here we analyze the species richness data for various countries in Latin America, using the above-mentioned altitudinal amplitude as a proxy. Habitat diversity played a role in tropical, but not in temperate countries. The reason may indicate that in the temperate countries only few orchid species grow in higher elevations, so an increase of altitudinal range of habitats there does not entail a corresponding increase of species richness there. Thus, especially in the tropics, efforts should be directed to preservation of protected areas in all altitudes, rather than to increase of existing reserve size only in areas that are not attractive for human development.

Key words: altitude, habitat diversity, species-area relationship

Introduction. Questions concerning species diversity have attracted ecologists for over a century. Recently this issue became even more important, because the diversity of life on Earth is in rapid decline (Dirzo & Raven 2003, Possingham & Wilson 2005). Therefore, one of the most pressing tasks facing the global conservation community is trying to understand the main factors determining diversity of species (Possingham & Wilson 2005) and identifying important areas for their conservation (Tsiftsis *et al.* 2011), as this is crucial for their survival. This especially holds for threatened groups such as orchids (Efimov 2011, Feldman & Prat 2011).

For any group of organisms, the most important determinants of species richness are thought to be area, latitude and energy availability (Schödelbauerová *et al.* 2009). Specifically, the increase in species richness with decreasing latitude from the poles to the tropics (Pianka 1966, Rohde 1992, Willig *et al.* 2003, Hillebrand 2004) was well documented and so was the increase in species richness with increasing area (Arrhenius 1921, Gleason 1922, Williamson 1988, Rosenzweig 1995). More recently, the amount of energy available (i.e. that which can be converted into biomass) for net primary productivity has been revealed to be also an important determinant of species richness (Wright 1983, Wylie & Currie 1993a, 1993b, Pelkey *et al.* 2000, Evans *et al.* 2005, Storch *et al.* 2005).

The relative importance of these factors was recently tested, using data on orchid species diversity in various countries in the world (Schödelbauerová *et al.* 2009) and it was found that size of the region considered is a better determinant of species diversity in orchids than its latitude.

However, the habitat heterogeneity hypothesis states that also an increase in habitat heterogeneity leads to an increase in species diversity (Cramer & Willig 2005). The effect of habitat diversity on species diversity was empirically supported by the

Received 3 May 2016; accepted for publication 30 July 2016. First published online: 30 August 2016.

TABLE 1. Sources for the numbers of orchid species countries from Central and South America.

Tropical America	
Belize	IUCN/SSC Orchid Specialist Group (2004)
Bolivia	Vasquez, Ch.R., Pierre, L.I., 2000. Orchids of Bolivia – Diversity and Conservation Status, vol. 1. Subtribu: Pleurothallidinae. Editorial F.A.N., Bolivia
Brazil	IUCN/SSC Orchid Specialist Group (2004)
Amazonia, Brazil	IUCN/SSC Orchid Specialist Group (2004)
State of Espirito Santo, Brazil	IUCN/SSC Orchid Specialist Group (2004)
Colombia	IUCN/SSC Orchid Specialist Group (2004)
Costa Rica	Dodson, C.H., Escobar, R., 1994. Native Ecuadorian Orchids I. Hola Colina, Medellin
Ecuador	Dodson, C.H., Escobar, R., 1994. Native Ecuadorian Orchids I. Hola Colina, Medellin
El Salvador	IUCN/SSC Orchid Specialist Group (2004)
French Guiana	IUCN/SSC Orchid Specialist Group (2004)
Guatemala	IUCN/SSC Orchid Specialist Group (2004)
Guyana	IUCN/SSC Orchid Specialist Group (2004)
Honduras	IUCN/SSC Orchid Specialist Group (2004)
Mexico	Espejo-Serna, A., López-Ferrari, A.R., 1998. Las monocotiledóneas mexicanas, una sinopsis florística. 1 Lista de Referencia. Parte VIII. Orchidaceae 2. Consejo de la Flora de México A.C., Universidad Autónoma Metropolitana Iztapalapa, CONABIO, D.F. México
Nicaragua	IUCN/SSC Orchid Specialist Group (2004)
Panama	Dodson, C.H., Escobar, R., 1994. Native Ecuadorian Orchids I. Hola Colina, Medellin
Peru	IUCN/SSC Orchid Specialist Group (2004)
Suriname	IUCN/SSC Orchid Specialist Group (2004)
Venezuela	IUCN/SSC Orchid Specialist Group (2004)
Temperate America	
Argentina	IUCN/SSC Orchid Specialist Group (2004)
Chile	IUCN/SSC Orchid Specialist Group (2004)
Paraguay	IUCN/SSC Orchid Specialist Group (2004)
Uruguay	IUCN/SSC Orchid Specialist Group (2004)

data on orchids in the Caribbean islands (Ackerman *et al.* 2007), but habitat heterogeneity has never been considered in the context of orchids growing on the mainland – e.g., various countries in a continent. The result obtained for islands (that increase in habitat heterogeneity leads to an increase in species diversity) cannot be automatically transferred to the case of a set of patches on the mainland (like various countries in a continent). The reason is that while islands are surrounded by a completely inhospitable area for orchids (sea), the area between individual patches on the mainland is covered by various types of habitat (usually called "matrix"), some of which ("hospitable patches") can be inhabited by the taxonomic group in question (orchids in this case). Therefore, these "hospitable patches" may act as stepping stones for migration of species between the patches studied. This also holds for passively migrating groups, like orchids. Their seeds originating from patch A may be blown by wind, germinate on these stepping stones and their descendant seeds may finally reach some other patch B. If the distance between patches A and B is large and therefore the likelihood that a seed originating from patch A will reach directly patch B, then the presence of the stepping stone increases the probability that species originating from patch A finally colonizes patch B via descendants of the seeds produced in patch A.

Table 2. Models tested, their parameters, and the corresponding t-, p- and R^2-values.

Model	**Coefficient**	**Value**	**t**	**p**	**R^2**
S = a*Alt + b*A +c	a	0.32	4.14	< 0.05	0.58
	b	0.0001	1.97	0.07	
	c	-198.13	-0.62	0.55	
S = a*Alt + b*ln A +c	a	0.28	2.89	< 0.05	0.52
	b	112.3	1.11	0.28	
	c	-1300.6	-1.14	0.27	
ln S = a*Alt + b*ln A +c	a	0.0003	4.15	< 0.05	0.69
	b	0.11	1.64	0.12	
	c	4.41	5.84	< 0.05	

Here we show our results on the combined effect of area and habitat diversity on orchid species diversity in various countries, using data from Central and South America. Our results therefore provide a comparison of the results already obtained in islands with those for mainland.

Methods. The numbers of orchid species (S) for 23 countries or parts thereof from Central and South America and their areas were obtained from published articles and floras (Table 1). These countries were divided into temperate (Argentina, Chile, Paraguay and Uruguay) and tropical ones (the remaining 19 countries). Area (A) and habitat diversity, estimated by the altitudinal difference between the highest and lowest point in the country (Alt), were then obtained from web sites of these countries or using Google Earth.

We used both univariate (power function for S = f(A) and exponential for S = f(Alt)) and 3 different linear bivariate models (see Table 2 for their list). Only univariate models were used for temperate countries, as for only 4 countries there was no point in fitting the data by bivariate models.

Results. We analysed 19 tropical countries and areas (parts of a country) and 4 temperate countries in Latin America. The species-area relationships for tropical and temperate countries are shown in Fig. 1. Area of the country explained 39% of the variability in temperate countries and 36% of the variability in tropical countries.

The dependence of the number of orchid species on habitat diversity approximated as the difference between the altitude of the highest and that of the lowest point in the country is shown in Fig. 2. For tropical countries, habitat diversity was even a better predictor of the number of orchid species than area, as it explained 64% of the variability. In the temperate countries, habitat diversity explained only 5% of the total variability.

Table 2 summarizes the models tested, their parameters, and the corresponding t-, p- and R^2-values. The coefficient *a* for habitat diversity, approximated as the difference between the altitude of the highest and that of the lowest point in the country was always significantly different from zero, as indicated by the p-value, so habitat heterogeneity should never be neglected. The R^2-values indicating the percentage of explained variability by the model show that the best model is the third one, i.e., ln S = a*Alt + b*ln A + c.

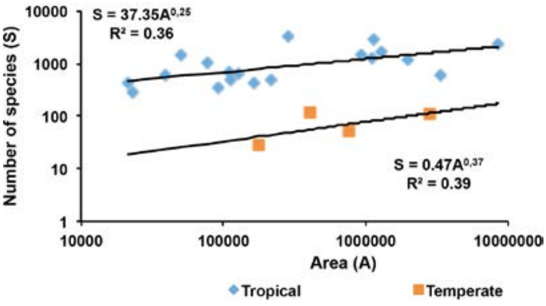


Figure 1. The species-area relationship, for temperate and tropical countries separately.

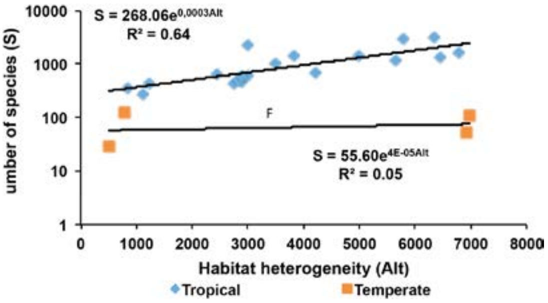


FIGURE 2.The dependence of the number of species on the altitudinal difference between the highest and lowest point in the country (Alt). Alt represents an estimate of habitat diversity in the country..

Discussion. The model ln S = a*Alt + b*ln A + c best fitted the data on orchid species richness, which only reconfirms the well known fact that a power function is the best one for fitting the species-area relationships. Not surprisingly, the effect of latitude is expressed by lower intercept in Fig. 1 for temperate countries. The importance of the slightly larger exponent for temperate countries cannot be overestimated because of the low number of these countries.

Habitat diversity, as expressed by the difference in altitudes between the highest peak and lowest point in the country considered, played a role in tropical, but not in temperate countries (Fig. 2). The reason may be that in temperate countries only few orchid species grow in higher elevations, so an increase of altitudinal range of habitats there does not entail a corresponding increase of species richness.

Both figures and the table indicate that the effect of habitat diversity was stronger than that of country size in tropical countries. Thus, especially in the tropics, efforts should be directed to preservation of protected areas in all altitudes, rather than to increase of existing reserve size only in areas that are not attractive for human development.

Altitudinal amplitude is used as a proxy for habitat here. Of course, with increasing altitude comes slightly increasing area compared to the basal area of the country. This could be a confounding factor in our study. However, first – the increase of area caused by habitat heterogeneity is relatively small (altitudinal differences per kilometre are usually in the range of several hundreds of meters at most). Second – even if we accept there is a positive correlation between area and habitat diversity per unit basal area, then this increase just becomes one of the components of the effect of habitat diversity. This is not to say that this effect should not be completely ignored, however.

Our results show a strong correlation with habitat diversity, which would suggest potentially narrow habitat ranges of species. This may have some implications particularly in the light of climate change: if temperature will increase, the species will most likely move upwards and if there is no space there (top of the mountain), the only chance for them to survive will be to move to another patch. Even if there will be space in higher elevations, the basic topological considerations imply that its amount may be lower.

The potentially narrow habitat ranges of species may also have important implications in protected area management: maintenance of present locations of species may then become very important, as other locations may be unsuitable because of this narrow habitat range.

ACKNOWLEDGEMENTS. This research was supported by the grant No. 14-36098G of the GA CR to PK and ZS and by the MSMT within the National Sustainability Program I (NPU I), grant number LO1415 to IT.

LITERATURE CITED

Ackerman, J.D., Trejo-Torres, J.C. & Crespo-Chuy, Y. (2007). Orchids of the West Indies: predictability of Diversity and endemism. *Journal of Biogeography,* 34, 779-786.

Arrhenius, O. (1921). Species and area. *Journal of Ecology,* 9, 95–99.

Cramer, M.J. & Willig, M.R. (2005). Habitat heterogeneity, species diversity and null models. *Oikos*, 108, 209-218.

Dirzo, R. & Raven, P.H. (2003). Global state of biodiversity and loss. *Annual Review of Environmental Research*, 28, 137-167.

Efimov, P.G. (2011). Revealing the decline and expansion of orchids of NW European Russia. *European Journal of Environmental Sciences*, 1(2), 7-17.

Evans, K.L., Greenwood, J.J.D. & Gaston, K.J. (2005). The roles of extinction colonization in generating species–energy relationships. *Journal of Animal Ecology*, 74, 498–507.

Feldman, D. & Prat, D. (2011). Conservation recommendations from a large survey of French orchids. *European Journal of Environmental Sciences*, 1(2), 8-27.

Gleason, H.A. (1922). On the relation between species and area. *Ecology*, 3, 158–162.

Hillebrand, H. (2004). On the generality of the latitudinal diversity gradient. *American Naturalist,* 163, 192–211.

Pelkey, N.W., Stoner, C.J. & Caro, T.M. (2000). Vegetation in Tanzania: assessing long term trends and effects of protection using satellite imagery. *Biological Conservation,* 94, 297-309.

Pianka, E.R. (1966). Latitudinal gradients in species diversity: a review of concepts. *American Naturalist,* 100, 33-46.

Possingham, H. P. & Wilson, K.A. (2005). Turning up the heat on hotspots. *Nature*, 436, 919-920.

Rohde, K. (1992). Latitudinal gradients in species diversity: the search for the primary cause. *Oikos*, 65, 514-527.

Rosenzweig, M.L. (1995). Species Diversity in Space and Time. Cambridge University Press, Cambridge.

Schödelbauerová, I., Roberts, D.L. & Kindlmann, P. (2009). Size of protected areas is the main determinant of species diversity in orchids. *Biological Conservation*, 142, 2329-2334.

Storch, D., Evans, K.L. & Gaston, K.J. (2005). The species-area-energy relationship. *Ecology Letters*, 8, 487-492.

Tsiftsis, S., Tsiripidis, I. & Trigas, R. (2011). Identifying important areas for orchid conservation in Crete. *European Journal of Environmental Sciences*, 1(2), 28-37.

Williamson, M.H. (1988). Relationship of species number to area, distance and other variables. In: A.A. Myers & P.S. Giller (eds.), *Analytical Biogeography* (pp. 91-115). London: Chapman and Hall.

Willig, M.R., Kaufman, D.M. & Stevens, R.D. (2003). Latitudinal gradients in biodiversity: pattern, process, scale, and synthesis. *Annual Review or Ecology and Systematics,* 34, 273-309.

Wright, D.H. (1983). Species-energy theory: an extension of species-area theory. *Oikos,* 41, 496-506.

Wylie, J.L. & Currie, D.J. (1993a). Species-energy theory and patterns of species richness: I. Patterns of bird, angiosperm, and mammal species richness on islands. *Biological Conservation,* 63, 137-144.

Wylie, J.L. & Currie, D.J. (1993b). Species-energy theory and patterns of species richness: II. Predicting mammal species richness on isolated nature reserves. *Biological Conservation,* 63, 145-148.

LANKESTERIANA 16(2): 299–305. 2016. doi: http://dx.doi.org/10.15517/lank.v16i2.26014

MAIN FUNGAL PARTNERS AND DIFFERENT LEVELS OF SPECIFICITY OF ORCHID MYCORRHIZAE IN THE TROPICAL MOUNTAIN FORESTS OF ECUADOR

Juan Pablo Suárez[1,3] & Ingrid Kottke[2]

[1] Departamento de Ciencias Naturales, Universidad Técnica Particular de Loja, San Cayetano Alto s/n
C.P. 11 01 608, Loja, Ecuador*

[2] Plant Evolutionary Ecology, Institute of Evolution and Ecology, Eberhard-Karls-University Tübingen,
Auf der Morgenstelle 1, 72076 Tübingen, Germany; retired

[3] Corresponding author: jpsuarez@utpl.edu.ec

Abstract. Orchids are a main component of the diversity of vascular plants in Ecuador with approximately 4000 species representing about 5.3% of the orchid species described worldwide. More than a third of these species are endemics. As orchids, in contrast to other plants, depend on mycorrhizal fungi already for seed germination and early seedling establishment, availability of appropriate fungi may strongly influence distribution of orchid populations. It is currently debated if green orchids depend on specific mycobionts or may be equally promoted by a broad spectrum of mycorrhizal fungi, discussion mostly based on data from temperate regions. Here we summarize results obtained from broad scale investigations in the tropical mountain rain forest of Ecuador revealing associations with members of Serendipitaceae (Sebacinales), Tulasnellaceae, Ceratobasidiaceae (Cantharellales), and Atractiellales. Recent molecular data show that these worldwide spread fungal groups have broad ecological implications and are specifically suited as mycorrhizal fungi of green orchids. We found that main fungal partners and different levels of specificity among orchids and their mycobionts in the tropical mountain forests correspond to findings in other biomes despite the large ecological differences.

Key words: Atractiellales, Ceratobasidiaceae, epiphytic orchids, mycobionts, Serendipitaceae, Tulasnellaceae

Introduction. Interaction between orchids and their mycorrhizal fungi is characterized by production of thousands of tiny seeds lacking carbohydrate reserves. Colonization of a seed by a suitable mycorrhizal fungus is, thus, vital for successful germination, growth and establishment of orchids in nature. The associations with mycorrhizal fungi remain when orchids become photosynthetic (Dearnaley *et al.* 2012). The extent to which mycobionts support growth of adult orchids in nature is unknown, but the maintenance of the mycobionts is, without doubt, crucial for their juveniles. It was shown that success of seedling establishment is affected by occurrence of mature plants, despite the easy far-distance transport of orchid seeds (Jacquemyn *et al.* 2007; Jersákovà & Malinová 2007; Riofrío *et al.* 2013). It is under current debate if orchid species depend on specific mycobionts or can equally or better be promoted by a broad spectrum of fungi (Kartzinel *et al.* 2013; Kottke *et al.* 2013; McCormick & Jacquemyn 2014). Narrow, specific association might be more efficient (Kiers *et al.* 2011) and favor propagation of the respective plant and mycobiont against competing species, while a broader mutualistic interaction might be safer in long term survival and distribution. Thus, knowledge on identity of fungal mycobionts and degree of specificity in symbioses is of interest to understand life history and distribution of orchids and a precondition for conservation efforts in such diverse places like the mountain forests of Ecuador where orchids, with approximately 4000 species, constitute about 25% of native vascular plants (Dodson 2005; Neill 2012). Comprehensive molecular studies on the mycobionts of terrestrial and epiphytic orchids were therefore carried out in the tropical mountain forest of Southern Ecuador (Kottke *et al.* 2010, 2013; Riofrío *et al.* 2013; Suárez *et al.* 2006; Suárez *et al.* 2008; Suárez

Received Received 3 May 2016; accepted for publication 12 July 2016. First published online: 30 August 2016.

et al. 2016). Results are summarized here and recent molecular phylogenetic and physiological data of the respective fungal groups are compiled to discuss the findings.

Main fungal partners associated with tropical orchids. The autotrophic terrestrial and epiphytic orchids in the tropical mountain rain forest were found associated with a limited range of Basidiomycota in the Serendipitaceae (Sebacinales), Tulasnellaceae and Ceratobasidiaceae (Cantharellales), and Atractiellomycetes (Atractiellales) (Kottke *et al.* 2010, 2013; Otero *et al.* 2002; Otero *et al.* 2007; Suárez *et al*. 2006, 2008, 2016). Tulasnellaceae were the most species rich and most abundant group. All of these fungal groups produce cryptic fruiting structures, which hinder their taxonomic resolution by morphological characters, but molecular approach gives well supported insights at high resolution level (Oberwinkler *et al*. 2006, 2013a, Oberwinkler *et al.* 2013b, 2014; Cruz *et al.* 2016). The nuclear ribosomal RNA locus, in particular the highly variable internal transcribed spacer region including 5.8S (nrITS-5.8S) is among the most widely used locus for phylogenetic studies in fungi and was recently proposed as the universal barcode region for fungi (Schoch *et al*. 2012). The nrDNA are multi-copy genes and therefore easily amplified from environmental samples. Large numbers of sequences were thus obtained from our samples and analyzed by molecular phylogenetic models including all available data from gene bank. Considering also literature on molecular systematics and whole genome studies of Agaricomycotina (Basidiomycota) we may now better understand why just these fungal groups became orchid mycobionts (Hibbet & Matheny 2009; Kohler *et al*. 2015).

Sebacinales —. Sebacinales are a basal lineage in Agaricomycotina (Basidiomycota) and include root endophytes, saprotrophic species and species forming mycorrhizal associations (Weiss *et al.* 2004). Recently, Sebacinales were divided in two sister families: Sebacinaceae, previously Sebacinales subgroup A, and Serendipitaceae, previously Sebacinales subgroup B (Weiss *et al.* 2016). Members of Sebacinaceae form ectomycorrhizae with diverse tree species (Avis *et al.* 2003; Glen *et al.* 2002; Kennedy *et al.* 2003; Shefferson *et al.* 2005; Tedersoo *et al.* 2006; Walker *et al.* 2005; Weiss *et al*. 2004) including tripartite associations with achlorophyllous and mixotrophic orchids (Julou *et al.* 2005; McKendrick *et al.* 2002; Selosse *et al.* 2004; Selosse *et al.* 2002; Taylor *et al.* 2003; Urban *et al.* 2003; Warcup 1971). Sebacinoid mycobionts from green orchids, including epiphytic orchids, appear in Serendipitaceae (Suárez *et al.* 2008). The same was found for Andean Ericaceae (Setaro *et al.* 2006a, 2006b).

Cantharellales —. Members of Tulasnellaceae and Ceratobasidiaceae are placed in Cantharellales, a basal lineage in Agaricomycotina (Basidiomycota), which contains nearly exclusively saprotrophs or mutualistic biotrophs (Hibbett *et al*. 2007, 2014; Veldre *et al*. 2013). Tulasnelloid fungi are world-wide spread, producing inconspicuous, resupinate basidiomata on rotten wood or bark of tree branches (Cruz *et al.* 2014; Cruz *et al*. 2016; Roberts 1999). Tulasnellaceae form mycorrhizae with a broad spectrum of plants, including mycorrhiza-like association with *Aneuraceae* (liverworts) thallus (Kottke *et al.* 2003; Krause *et al.* 2011) tripartite associations with the heterotrophic liverwort species *Cryptothallus mirabilis* and with surrounding trees as ectomycorrhizal partners (Bidartondo *et al.* 2003). *Tulasnella* was also found associated with *Graffenrieda emarginata* (*Melastomatacecae*) forming a superficial layer on arbuscular mycorrhizas (Haug *et al*. 2004). Most importantly, however, *Tulasnella species* are the most frequent and widespread mycobionts of autotrophic orchids (reviewed by Kottke & Suárez 2009 and Dearnaley *et al.* 2012). While only few named *Tulasnella* species are reported as forming mycorrhiza with orchids, molecular phylogeny revealed a large number of genotypes, but also inconsistencies in species concepts and taxonomy challenging comparative ecological studies (Cruz *et al.* 2011, 2014, 2016). These recent studies on fresh samples of basidiomata showed however, that molecular approach using the ITS-5.8 region and a threshold of 4 % up to 8 % variability gives well supported clades considered as corresponding to species level.

The genera *Ceratobasidium* and *Thanatephorus* along with their *Rhizoctonia* anamorphs form a group of closely related fungal taxa in the family Ceratobasidiaceae. A recent phylogenetic analysis revealed ten groups within the Ceratobasidiaceae (González *et al.* 2016). Species within the *Ceratobasidium-Thanatephorus* complex are known as crop pathogens but also as forming mycorrhizae with orchids and trees (Tedersoo *et al.* 2010). Delineating species in these groups has been problematic, efforts to solve this situation included anastomosis groups and molecular data (Oberwinkler *et al.* 2013b). Studies reporting the presence of members of Ceratobasidiaceae from tropical orchids remain scarce. Using isolation-dependent methods orchid species *Coppensia doniana, Tolumnia variegata, Ionopsis utricularioides* and *Psygmorchis pusilla* (tribe Cymbidieae) were shown to be predominantly associated to Ceratobasidiaceae (Valadares *et al.* 2015; Otero *et al.* 2002, 2004). However, using isolation-independent methods, members of Ceratobasidiaceae were absent in species of *Pleurothallis, Stelis* and *Epidendrum* (tribe Epidendreae) (Suárez *et al.* 2006, 2008; Kottke *et al.* 2010; Riofrío *et al.* 2013).

Atractiellales—. Atractiellales belong to the subphylum Pucciniomycotina (Rust fungi), which comprises mainly parasites and to a lesser extent presumed saprophytes (Aime *et al.* 2006). The phylogenetic position of the mycobionts among potential saprophytes may indicate physiological flexibility from saprophytism to mutualism, as required for orchid mycobionts (Rasmussen & Rasmussen 2009). So far only three genotypes (operational taxonomic units, OTUs) of Atractiellomycetes were shown by combined ultrastructural and molecular investigations to form mycorrhiza with terrestrial and epiphytic orchids (Kottke *et al.* 2010; Riofrío *et al.* 2013; Suárez *et al.* 2016). Experimental proof for nutritional support of protocorms is, however, still lacking.

Orchid-mycobiont interaction with different levels of specificity in the tropical mountain forest. Although a high number of orchid species have been recorded, studies on their mycorrhizal fungi are still scarce (Dearnaley *et al.* 2012), and in most cases, only few individuals per population have been sampled. Factors as orchid recruitment, seed dispersal limitations and availability of suitable fungal partner are affecting the distribution of orchids at regional and local scales and may disguise specificities among plants and fungi. Our studies were concentrated on the conditions of tropical mountain rain forest where intensive sampling was carried out on epiphytic and terrestrial species. Molecular data revealed narrow preferences to broad sharing of partners.

In a pioneer study by Suárez *et al.* (2006) in the mountain forests of Zamora-Chinchipe of Ecuador, using a combination of fungal isolation and fungal-independent investigations, differences in the number of mycorrhizal fungal partners were found among three *Stelis* and one *Pleurothallis* species, two closely related genera (subtribe Pleurothallidinae, tribe Epidendreae). Preferences were evident in case of *S. concinna,* where the highest number of plant individuals was investigated, but the lowest number of fungi was detected. *Stelis concinna* was associated with only one member of Serendipitaceae and two members of Tulasnellaceae, two of these showing a wide elevation distribution range (Suárez *et al.* 2006). Beside these preferences, a broad sharing of mycobionts was observed among *Stelis* and *Pleurothallis* species (Suárez *et al.* 2008) potentially explained by their close phylogenetic relationship (Pridgeon, Solano & Chase 2001).

Riofrío *et al.* (2013) carried out a study in a nearby site, focusing on within-population variation of mycorrhizal associations of the epiphytic orchid *Epidendrum rhopalostele* (subtribe Laeliinae, tribe Epidendreae). Individuals were associated with only two different clades of closely related *Tulasnella.* The two clades were spatially randomly distributed showing no segregation patterns, both clades were related to the *Tulasnella* isolated from *Stelis* and *Pleurothallis* by Suárez *et al.* (2006), but corresponded to distinct genotypes.

Kottke *et al.* (2010) showed that three Atractiellales genotypes, closely related to *Infundibura,* formed mycorrhizas with many terrestrial and epiphytic orchid species in the Andean tropical forest and regenerating habitats. Similar broad sharing was found for *Tulasnella* and *Seredipita* genotypes from the same area (Kottke *et al.* 2013).

A study carried out near the town of Baños, Tungurahua province, far from Zamora-Chinchipe area, revealed that *Teagueia* morphospecies (subtribe Pleurothallidinae, tribe Epidendreae) were associated with members of Tulasnellaceae and Atractiellales. The phylogenetic analysis of ITS-5.8S sequences of members of Tulasnellaceae showed sequences in four clades. Sequences from the previous study by Suárez *et al*. (2006) are closely related to these clades. The obtained Atractiellales sequences were identical to "phylotype I" as found by Kottke *et al*. (2010) from the previous study in Zamora-Chinchipe, Ecuador. Results show up to three different phylogenetic species of mycobionts associated to one *Teagueia* species suggesting high potential for sharing mycobionts among *Teagueia* spp. All the detected mycobionts had wide geographical distribution.

Discussion. The molecular phylogenetic based investigations of orchid mycobionts in the tropical mountain rain forest area of Southern Ecuador revealed the well established fungal groups, Serendipidaceae, Tulasnellaceae and Ceratobasidiaceae. Additionally, Atractiellales were found as widespread mycobionts. The latter can be found in some data sets of previous investigations, but were not seriously considered before. Although we cannot definitely exclude that further fungal groups may be detected in future, we may ask why just members of these few fungal families are suitable mycobionts of green orchid. The vast majority of plants form mycorrhizae with Glomeromycota, obligate symbionts with no access to extraradical sugars. Thus, these mycobionts are unsuitable to feed the orchid protocorm. Mycobionts in Agaricomycetes, however, derived from saprotrophic relatives multiple times and the orchid mycorrhizal groups, in basal position of Agaricomycetes, preserved some genes for decay enzymes (Hibbett *et al*. 2007; Kohler *et al*. 2015). Preservation of the respective genes means sufficient organic matter decay for acquiring carbon and nitrogen to feed the orchid protocorm without attacking living cells in a parasitic manner. According with these capabilities, these fungi are prepared to grow on tree bark (Kartzinel *et al*. 2013) and may additionally profit from the water-retention capacity of the velamen covering roots of epiphytic orchids. The particular conditions in the epiphytic habitat may have potentially supported adaptations to the specific groups of mycorrhizal fungi. The life history sets Orchidaceae apart from all other Monocotyledonae and was obviously facilitated by switching from Glomeromycota to mainly these Agaricomycetes in Basidiomycota as mycorrhizal fungi. We may consider this switch as a synapomorphy of the family (Yukawa, Ogura-Tsujita, Shefferson & Yokoyama. 2009).

Another question under debate concerns about potential specificity among orchids and mycobionts. Most investigations focused on rather species poor orchid populations in temperate areas (McCormick & Jacquemyn 2014). It was generally found that orchid rarity was unrelated to specificity, but coexisting, terrestrial orchid populations were associated with distinct mycobiont communities. In case of tropical epiphytic orchids, availability of appropriate fungi may be especially critical for plant survival under the restricted conditions of shortage of water or organic and inorganic nutrients (Zotz & Hietz 2001; Martos *et al*. 2012; Dearnaley *et al*. 2012). Fungi may be rare to meet on tree branches and stems, potentially restricted to species adapted to these niches. Accordingly, Martos *et al*. (2012) found significant differences among epiphytic and terrestrial orchid mycobionts in old world tropical Reunion island. We carried out a comparatively large-scale survey in the new world tropical forest of the Ecuadorian Andes on epiphytic and terrestrial orchids. Our results showed different levels of specificity, ranging from unspecific, multi-species networks to narrow orchid clades associated with few world-wide spread *Tulasnella* genotypes, and to narrow species-species associations along an elevation gradient or among dense populations of an epiphytic orchid. Species richness, environmental conditions but also sampling efforts and methods of fungal identification may still bias a clear statement on narrow specificity in the wet tropical habitat. More likely, sharing of mycobionts will improve the rate of successful germination of orchid seeds and also promote co-existence of closely related species as observed in the tropical montane rain forest (Kottke *et al*. 2013).

ACKNOWLEDGMENTS. The Universidad Técnica Particular de Loja- UTPL and the Deutsche Forschungsgemeinschaft-DFG financed this research. We thank the Reserva Biológica San Francisco for providing research facilities.

Literature Cited

Aime, M. C., Matheny, P. B., Henk, D. A., Frieders, E. M., Nilsson, R. H., Piepenbring, M., *et al.* (2006). An overview of the higher level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences. *Mycologia*, 98(6), 896–905.

Avis, P., McLaughlin, D., Dentinger, B. & Reich, P. (2003). Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. *New Phytologist*, 160(1), 239–253.

Bidartondo, M. I., Bruns, T. D., Weiss, M., Sergio, C. & Read, D. J. (2003). Specialized cheating of the ectomycorrhizal symbiosis by an epiparasitic liverwort. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 270(1517), 835–842. http://doi.org/10.1098/rspb.2002.2299

Cruz, D., Suárez, J. P. & Piepenbring, M. (2016). Morphological revision of Tulasnellaceae, with two new species of *Tulasnella* and new records of *Tulasnella* spp. for Ecuador. *Nova Hedwigia*, 102(3-4), 279-338.

Cruz, D., Suárez, J. P., Kottke, I. & Piepenbring, M. (2014). Cryptic species revealed by molecular phylogenetic analysis of sequences obtained from basidiomata of *Tulasnella*. *Mycologia*, 106(4), 708–722. http://doi.org/10.3852/12-386

Cruz, D., Suárez, J. P., Kottke, I., Piepenbring, M. & Oberwinkler, F. (2011). Defining species in *Tulasnella* by correlating morphology and nrDNA ITS-5.8S sequence data of basidiomata from a tropical Andean forest. *Mycological Progress*, 10(2), 229–238. http://doi.org/10.1007/s11557-010-0692-3

Dearnaley, J., Martos, F. & Selosse, M. A. (2012). Orchid Mycorrhizas: Molecular Ecology, Physiology, Evolution and Conservation Aspects. In: B. Hock (Ed.), *The Mycota IX: Fungal associations* (pp. 207-230). Berlin, Heidelberg: Springer.

Dodson, C. (2005). Native Ecuadorian Orchids, vol IV. 665 pp.

Glen, M., Tommerup, I., Bougher, N. & O'Brien P.A. (2002) Are Sebacinaceae common and widespread ectomycorrhizal associates of Eucalyptus species in Australian forests? *Mycorrhiza,* 12, 243–247.

González, D., Rodriguez-Carres, M., Boekhout, T., Stalpers, J., Kuramae, E. E., Nakatani, A. K., *et al.* (2016). Phylogenetic relationships of *Rhizoctonia* fungi within the Cantharellales. *Fungal Biology*, 120(4), 603–619.

Haug, I., Lempe, J., Homeier, J., Weiss, M., Setaro, S. D., Oberwinkler, F. & Kottke, I. (2004). *Graffenrieda emarginata* (Melastomataceae) forms mycorrhizas with Glomeromycota and with a member of the *Hymenoscyphus ericae* aggregate in the organic soil of a neotropical mountain rain forest. *Canadian Journal of Botany*, 82(3), 340–356.

Hibbett, D. S., Bauer, R., Binder, M., Giachini, A. J., Hosaka, K., Justo, A., *et al.* (2014). Agaricomycetes. In: *Systematics and Evolution* (pp. 373–429). Berlin, Heidelberg: Springer. http://doi.org/10.1007/978-3-642-55318-9_14

Hibbett, D. S., Binder, M., Bischoff, J. F., Blackwell, M., Cannon, P. F., Eriksson, O. E., *et al.* (2007). A higher-level phylogenetic classification of the Fungi. *Mycological Research*, 111(Pt 5), 509–547. http://doi.org/10.1016/j.mycres.2007.03.004

Hibbett, D. & Matheny, P. (2009). The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses. *BMC Biology*, 7(1), 13.

Jacquemyn, H., Brys, R., Vandepitte, K., Honnay, O., Roldán Ruiz, I. & Wiegand, T. (2007). A spatially explicit analysis of seedling recruitment in the terrestrial orchid *Orchis purpurea*. *New Phytologist*, 176(2), 448–459.

Jersákovà, J. & Malinová, T. (2007). Spatial aspects of seed dispersal and seedling recruitment in orchids. *New Phytologist*, 176, 237–241.

Julou, T., Burghardt, B., Gebauer, G., Berveiller, D., Damesin, C. & Selosse, M. A. (2005). Mixotrophy in orchids: insights from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasonium*. *New Phytologist*, 166(2), 639–653. http://doi.org/10.1111/j.1469-8137.2005.01364.x

Kartzinel, T. R., Trapnell, D. W. & Shefferson, R. P. (2013). Highly diverse and spatially heterogeneous mycorrhizal symbiosis in a rare epiphyte is unrelated to broad biogeographic or environmental features. *Molecular Ecology*, 22(23), 5949–5961. http://doi.org/10.1111/mec.12536

Kennedy, P. G., Izzo, A. & Bruns, T. D. (2003). There is high potential for the formation of common mycorrhizal networks between understorey and canopy trees in a mixed evergreen forest. *Journal of Ecology*, 91(6), 1071–1080.

Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E. *et al.* (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science,* 333, 880–882.

Kohler, A., Kuo, A., Nagy, L. G., Morin, E., Barry, K. W., Buscot, F., *et al.* (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics*, 47(4), 410–415. http://doi.org/10.1038/ng.3223

Kottke, I., Setaro, S., Haug, I., Herrera, P., Cruz, D., Fries, A., Gawlik, J., Homeier, J., Werner, F.A., Gerique, A. & Suárez, J.P. (2013) Mycorrhiza networks promote biodiversity and stabilize the tropical mountain rain forest ecosystem: Perspectives for understanding complex communities. In: *Ecological Studies* (Vol. 221, pp. 187–203). Berlin, Heidelberg: Springer.

Kottke, I. & Suárez, J.P. (2009) Mutualistic, root-inhabiting fungi of orchids identification and functional types. In: A.M. Pridgeon & JP Suárez (Ed.), *Proceedings of the Second Scientific Conference on Andean Orchids* (pp. 84–99). Loja, Ecuador: Universidad Técnica Particular de Loja.

Kottke, I., Suárez, J.P., Herrera, P., Cruz, D., Bauer, R., Haug, I. & Garnica, S. (2010) Atractiellomycetes belonging to the 'rust' lineage (Pucciniomycotina) form mycorrhizae with terrestrial and epiphytic Neotropical orchids. *Proceedings of the Royal Society of London, Series B: Biological Sciences,* 277, 1289-1298. doi: 10.1098/repb.2009.1884

Kottke, I., Beiter, A., Weiss, M., Haug, I., Oberwinkler, F. & Nebel, M. (2003). Heterobasidiomycetes form symbiotic associations with hepatics: Jungermanniales have sebacinoid mycobionts while *Aneura pinguis* (Metzgeriales) is associated with a *Tulasnella* species. *Mycological Research*, 107(Pt 8), 957–968.

Krause, C., Garnica, S., Bauer, R. & Nebel, M. (2011). Aneuraceae (Metzgeriales) and tulasnelloid fungi (Basidiomycota): a model for early steps in fungal symbiosis. *Fungal Biology*, 115(9), 839–851. http://doi.org/10.1016/j.funbio.2011.06.013

Martos, F., Munoz, F., Pailler, T., Kottke, I., Gonneau, C. & Selosse, M. A. (2012). The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. *Molecular Ecology*, 21(20), 5098–5109. http://doi.org/10.1111/j.1365-294X.2012.05692.x

McCormick, M. K. & Jacquemyn, H. (2014). What constrains the distribution of orchid populations? *New Phytologist*, 202(2), 392–400. http://doi.org/10.1111/nph.12639

McKendrick, S., Leake, J., Taylor, D. L. & Read, D. J. (2002). Symbiotic germination and development of the myco-heterotrophic orchid *Neottia nidus-avis* in nature and its requirement for locally distributed *Sebacina* spp. *New Phytologist*, 154(1), 233–247.

Neill D (2012). ¿Cuántas especies nativas de plantas vasculares hay en Ecuador? *Revista amazónica: Ciencia y Tecnología,* 1,70–83.

Oberwinkler, F., Riess, K., Bauer, R., & Garnica, S. (2014). Morphology and molecules: the Sebacinales, a case study. *Mycological Progress*, 13(3), 445–470. http://doi.org/10.1007/s11557-014-0983-1

Oberwinkler, F., Riess, K., Bauer, R., Selosse, M. A., Weiss, M., Garnica, S. & Zuccaro, A. (2013a). Enigmatic Sebacinales. *Mycological Progress*, 12(1), 1–27. http://doi.org/10.1007/s11557-012-0880-4

Oberwinkler, F., Riess, K., Bauer, R., Kirschner, R. & Garnica, S. (2013b) Taxonomic re-evaluation of the *Ceratobasidium-Rhizoctonia* complex and *Rhizoctonia butinii*, a new species attacking spruce. *Mycological Progress,* 12, 763-776.

Oberwinkler, F., Kirschner, R., Arenal, F., Villarreal, M., Rubio, V., Begerow, D. & Bauer, R. (2006) Two new members of the Atractiellales: *Basidiopycnis hyalina* and *Proceropycnis pinicola*. *Mycologia,* 98, 637-649.

Otero, J. T., Ackerman, J. D. & Bayman, P. (2002). Diversity and host specificity of endophytic Rhizoctonia-like fungi from tropical orchids. *American Journal of Botany*, 89(11), 1852–1858. http://doi.org/10.3732/ajb.89.11.1852

Otero, J. T., Flanagan, N. S., Herre, E. A., Ackerman, J. D. & Bayman, P. (2007). Widespread mycorrhizal specificity correlates to mycorrhizal function in the neotropical, epiphytic orchid *Ionopsis utricularioides* (Orchidaceae). *American Journal of Botany*, 94(12), 1944–1950. http://doi.org/10.3732/ajb.94.12.1944

Otero, J. T., Ackerman, J. D. & Bayman, P. (2004). Differences in mycorrhizal preferences between two tropical orchids. *Molecular Ecology*, 13(8), 2393–2404. http://doi.org/10.1111/j.1365-294X.2004.02223.x

Pridgeon, A., Solano, R. & Chase, M. W. (2001). Phylogenetic relationships in Pleurothallidinae (Orchidaceae): combined evidence from nuclear and plastid DNA sequences. *American Journal of Botany*, 88(12), 2286–2308.

Rasmussen, H. N. & Rasmussen, F. N. (2009). Orchid mycorrhiza: implications of a mycophagous life style. *Oikos*, 118(3), 334–345. http://doi.org/10.1111/j.1600-0706.2008.17116.x

Riofrío, M. L., Cruz, D., Torres, E., De la Cruz, M., Iriondo, J. M. & Suárez, J. P. (2013). Mycorrhizal preferences and fine spatial structure of the epiphytic orchid *Epidendrum rhopalostele*. *American Journal of Botany*, 100(12), 2339–2348.

Roberts, P. (1999). Rhizoctonia-forming Fungi: a taxonomic guide. Royal Botanic Gardens, Kew.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., *et al.* (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America*, 109(16), 6241–6246. http://doi.org/10.1073/pnas.1117018109

Selosse, M. A., Faccio, A., Scappaticci, G. & Bonfante, P. (2004). Chlorophyllous and achlorophyllous specimens of *Epipactis microphylla* (Neottieae, Orchidaceae) are associated with ectomycorrhizal Septomycetes, including Truffles. *Microbial Ecology*, 47(4), 416–426.

Selosse, M. A., Weiss, M., Jany, J. & Tillier, A. (2002). Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) L.C.M. Rich. and neighbouring tree ectomycorrhizae. *Molecular Ecology*, 11(9), 1831–1844.

Setaro, S. D., Kottke, I. & Oberwinkler, F. (2006a). Anatomy and ultrastructure of mycorrhizal associations of neotropical Ericaceae. *Mycological Progress*, 5(4), 243–254.

Setaro, S. D., Weiss, M., Oberwinkler, F. & Kottke, I. (2006b). Sebacinales form ectendomycorrhizas with *Cavendishia nobilis*, a member of the Andean clade of Ericaceae, in the mountain rain forest of southern Ecuador. *New Phytologist*, 169(2), 355–365. http://doi.org/10.1111/j.1469-8137.2005.01583.x

Shefferson, R. P., Weiss, M., Kull, T. & Taylor, D. L. (2005). High specificity generally characterizes mycorrhizal association in rare lady's slipper orchids, genus *Cypripedium*. *Molecular Ecology*, 14(2), 613–626. http://doi.org/10.1111/j.1365-294X.2005.02424.x

Suárez, J. P., Weiss, M., Abele, A., Garnica, S., Oberwinkler, F. & Kottke, I. (2006). Diverse tulasnelloid fungi form mycorrhizas with epiphytic orchids in an Andean cloud forest. *Mycological Research*, 110, 1257–1270. http://doi.org/10.1016/j.mycres.2006.08.004

Suárez, J. P., Weiss, M., Abele, A., Oberwinkler, F. & Kottke, I. (2008). Members of Sebacinales subgroup B form mycorrhizae with epiphytic orchids in a neotropical mountain rain forest. *Mycological Progress*, 7(2), 75–85. http://doi.org/10.1007/s11557-008-0554-4

Suárez, J. P., Eguiguren, J. S., Herrera, P. & Jost, L. (2016). Do mycorrhizal fungi drive speciation in *Teagueia* (Orchidaceae) in the upper Pastaza watershed of Ecuador? *Symbiosis*, 69(3), 161–168. http://doi.org/10.1007/s13199-016-0399-6

Taylor, D. L., Bruns, T. D., Szaro, T. & Hodges, S. (2003). Divergence in mycorrhizal specialization within *Hexalectris spicata* (Orchidaceae), a nonphotosynthetic desert orchid. *American Journal of Botany*, 90(8), 1168–1179.

Tedersoo, L., May, T. W. & Smith, M. E. (2010). Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza*, 20(4), 217–263. http://doi.org/10.1007/s00572-009-0274-x

Tedersoo, L., Suvi, T., Larsson, E. & Kõljalg, U. (2006). Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. *Mycological Research*, 110(6), 734–748.

Urban, A., Weiss, M. & Bauer, R. (2003). Ectomycorrhizas involving sebacinoid mycobionts. *Mycological Research*, 107(1), 3–14.

Valadares, R., Otero, J. T., Correa-Pereira, M. & Cardoso, E. (2015). The epiphytic orchids *Ionopsis utricularioides* and *Psygmorchis pusilla* associate with different *Ceratobasidium* lineages at Valle del Cauca, Colombia. *Acta Botanica Brasilica,* 29(1), 40-44.

Veldre, V., Abarenkov, K., Bahram, M., Martos, F., Selosse, M. A., Tamm, H., *et al*. (2013). Evolution of nutritional modes of Ceratobasidiaceae (Cantharellales, Basidiomycota) as revealed from publicly available ITS sequences. *Fungal Ecology*, 6(4), 256–268. http://doi.org/10.1016/j.funeco.2013.03.004

Walker, J. F., Miller, O. K. & Horton, J. L. (2005). Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the southern Appalachian Mountains. *Molecular Ecology*, 14(3), 829–838. http://doi.org/10.1111/j.1365-294X.2005.02455.x

Warcup, J. (1971). Specificity of mycorrhizal association in some Australian terrestrial orchids. *New Phytologist*, 70(1), 41–46.

Weiss, M., Selosse, M. A., Rexer, K., Urban, A. & Oberwinkler, F. (2004). Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. *Mycological Research*, 108(9), 1003–1010.

Weiss, M., Waller, F., Zuccaro, A. & Selosse, M-A. (2016) Sebacinales - one thousand and one interactions with land plants. *New Phytologist*, 211(1), 20-40.

Yukawa, T., Ogura-Tsujita, Y., Shefferson, R. P. & Yokoyama, J. (2009). Mycorrhizal diversity in Apostasia (Orchidaceae) indicates the origin and evolution of orchid mycorrhiza. *American Journal of Botany*, 96(11), 1997–2009. http://doi.org/10.3732/ajb.0900101

Zotz, G. & Hietz, P. (2001). The physiological ecology of vascular epiphytes: current knowledge, open questions. *Journal of Experimental Botany*, 52(364), 2067–2078.