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

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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. Pleurothallidiniae y Laeliinae son los grupos más ricos en especies y contienen la mayor géneros: Lepanthes, Pleurothallis, Stelis y...
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 from latest 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
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.

<table>
<thead>
<tr>
<th></th>
<th>Costa Rica</th>
<th>Panama</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genera</td>
<td>199</td>
<td>187</td>
<td>211</td>
</tr>
<tr>
<td>Species</td>
<td>1574</td>
<td>1372</td>
<td>2012</td>
</tr>
<tr>
<td>Endemics</td>
<td>485 (30.8)</td>
<td>299 (21.8)</td>
<td>784</td>
</tr>
</tbody>
</table>

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 biogeographic relationship could be to find species with a high percentage of endemic species in both countries and compare their distribution. One could find 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 2. The most diverse genera in Costa Rica and Panama. Number of species and percentage relative to the total of species of each genus.

<table>
<thead>
<tr>
<th></th>
<th>Costa Rica</th>
<th>Panama</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidendrum</td>
<td>207 (13.8)</td>
<td>Epidendrum</td>
<td>221 (14.7)</td>
</tr>
<tr>
<td>Stelis</td>
<td>88 (11.0)</td>
<td>Lepanthes</td>
<td>151 (13.5)</td>
</tr>
<tr>
<td>Lepanthes</td>
<td>66 (5.9)</td>
<td>Stelis</td>
<td>103 (12.9)</td>
</tr>
<tr>
<td>Pleurothallis</td>
<td>54 (6.8)</td>
<td>Camaridium</td>
<td>48 (32.0)</td>
</tr>
<tr>
<td>Camaridium</td>
<td>48 (32.0)</td>
<td>Pleurothallis</td>
<td>48 (6.0)</td>
</tr>
<tr>
<td>Scaphyglottis</td>
<td>39 (35.5)</td>
<td>Specklinia</td>
<td>44 (44.0)</td>
</tr>
<tr>
<td>Sobralia</td>
<td>39 (17.7)</td>
<td>Scaphyglottis</td>
<td>38 (34.5)</td>
</tr>
<tr>
<td>Specklinia</td>
<td>34 (34.0)</td>
<td>Sobralia</td>
<td>38 (17.3)</td>
</tr>
<tr>
<td>Oncidium</td>
<td>32 (8.0)</td>
<td>Telipogon</td>
<td>37 (14.8)</td>
</tr>
<tr>
<td>Dichaea</td>
<td>26 (26.0)</td>
<td>Masdevallia</td>
<td>34 (3.8)</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th></th>
<th>Costa Rica</th>
<th>Panama</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Telipogon</td>
<td>83.8</td>
<td>Telipogon</td>
<td>73.9</td>
</tr>
<tr>
<td>Lepanthes</td>
<td>67.5</td>
<td>Pleurothallis</td>
<td>42.6</td>
</tr>
<tr>
<td>Sobralia</td>
<td>39.5</td>
<td>Sobralia</td>
<td>42.6</td>
</tr>
<tr>
<td>Masdevallia</td>
<td>38.2</td>
<td>Masdevallia</td>
<td>34.6</td>
</tr>
<tr>
<td>Epidendrum</td>
<td>36.2</td>
<td>Lepanthes</td>
<td>31.5</td>
</tr>
<tr>
<td>Stelis</td>
<td>35.9</td>
<td>Stelis</td>
<td>33.3</td>
</tr>
<tr>
<td>Specklinia</td>
<td>31.8</td>
<td>Epidendrum</td>
<td>23.1</td>
</tr>
<tr>
<td>Pleurothallis</td>
<td>31.3</td>
<td>Camaridium</td>
<td>16.7</td>
</tr>
<tr>
<td>Camaridium</td>
<td>29.2</td>
<td>Specklinia</td>
<td>20.6</td>
</tr>
</tbody>
</table>
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 Hågsater 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

**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*.
number of species recorded in the Isthmus (Pupulin & Bogarin, 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, Bogarin 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 Bogarin 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, Bogarin 2007, Pupulin & Bogarin 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., Fernandeza Lindl. and Pterichis Lindl. that almost reach their northernmost distribution in the Cordillera de Talamanca (Bogarin et al. 2014b).

About 10 genera are present in Panama but not in Costa Rica. These genera have a strong South American relationship: Discyphus Schltr., Eloyella P.Oritz, Koellensteinia Rchb.f., Neomoorea Rolfe, Rudolfiella Hoehe 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 (Bogarin 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.Rehm., 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 (Bogarin 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

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

<table>
<thead>
<tr>
<th>Genera</th>
<th>Species in the Isthmus</th>
<th>% endemic species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepanthes</td>
<td>155</td>
<td>90.12</td>
</tr>
<tr>
<td>Epidendrum</td>
<td>133</td>
<td>46.18</td>
</tr>
<tr>
<td>Stelis</td>
<td>60</td>
<td>43.80</td>
</tr>
</tbody>
</table>
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.
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 Cecidomyiidae 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 (Dracanthes (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 and varies 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 (Pringeon, Solano-Gómez & Chase, 2001). Our preliminary phylogenetic analysis support the monophyly of Lepanthes. It is important to note that the genus Neoooreophilus Archila, which is closely related to Andinia (Luer) Luer and not to Lepanthes, seems to be a case of convergence (Fig. 8). Neoooreophilus species have a similar flower morphology as Lepanthes, and there is some evidence of its pollination by pseudocopulation (S. Vieira-Uribe, pers. comm. 2015). Neoooreophilus 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
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 *Bradygia* 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 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.
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).

Sciurids 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.

Sciurid 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. Sciuridae 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 Sciuridae 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 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.

Figure 8. Floral convergence among the species of Neoreophilus (A, B) and Lepanthes (C).
bottlenecks and genetic drift deserve 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**


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

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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, semi-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...
see Koch et al. 2013, Molineros-Hurtado et al. 2014, Pancaro 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-Valdies (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 colombiana, 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 reintroductions.
Vanilla species | Current known or Historical Distribution in Colombia | Citation for presence in Colombia
---|---|---
Vanilla bicolor Lindl. | Chocó | Molineros-Hurtado, 2012; Molineros et al., unpub. data
Vanilla calyculata Schltr. | Andean-Valle del Cauca | Molineros-Hurtado, 2012; Molineros et al., unpub. data
Vanilla colombiana Rolfe | Andean-Valle del Magdalena (historical) | Soto Arenas, 2010
Vanilla cribbiana Soto Arenas | Chocó | Molineros-Hurtado 2012; Molineros et al., unpub. data
Vanilla dressleri Soto Arenas | Chocó | Molineros-Hurtado, 2012; Molineros et al., unpub. data
Vanilla espondae Soto Arenas | Andean-Valle del Magdalena (historical) | Soto Arenas, 2010
Vanilla hebergeri Hawkes | Chocó | Molineros-Hurtado et al., unpub. data
Vanilla hostmanii Rolfe | Amazonia | Molineros-Hurtado et al., unpub. 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., unpub. 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., unpub. data
Vanilla planifolia Andrews | Chocó | Molineros-Hurtado 2012; Molineros et al., unpub. data
Vanilla pompona Schiede | Andean-Valle del Cauca | Molineros et al., unpub. data
Vanilla riberoi Hoehne | Amazonia (historical) | Soto Arenas, 2010
Vanilla rivsii Molineros-Hurtado et al | Chocó | Molineros-Hurtado, 2012; Molineros et al., unpub. data
Vanilla sprucei Rolfe | Amazonia (historical) | Soto Arenas, 2010
Vanilla trigonocarpa Hoehne | Chocó | Molineros-Hurtado 2012; Molineros et al., unpub. data

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

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
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 Tumbe-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.

<table>
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<tr>
<th>Political strategies</th>
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<tr>
<td>Evaluate the status of the whole of the genus <em>Vanilla</em> as exempt from CITES Appendix II.</td>
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<tr>
<td>Evaluate all species of <em>Vanilla</em> at the national level for inclusion in the IUCN Red List of Threatened Species.</td>
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<td>Consider special National protection for native <em>Vanilla</em> species.</td>
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<tr>
<td>Develop strategies to enforce existing national and international conservation legislation, including forensic genetic tools.</td>
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<td>Certification of authorized sustainable sources of <em>Vanilla</em> germplasm for commercialization.</td>
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<th>In situ conservation actions</th>
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<tr>
<td>Gap Analysis to ensure inclusion of <em>in situ</em> <em>Vanilla</em> populations in protected area networks, as well as maximum coverage of genetic diversity in <em>ex situ</em> collections.</td>
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<tr>
<td>Research program on biogeography, genetic diversity, ecology, phytochemistry and physiology of <em>Vanilla</em> species in natural habitat.</td>
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<tr>
<td>Development of DNA Barcodes for native <em>Vanilla</em> species, and further genetic profiling for forensic applications.</td>
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<tr>
<td>Develop a strategy for population restoration, including population augmentation, and reintroductions, both within and beyond natural ranges.</td>
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<th>Ex situ conservation actions</th>
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<td>Establishment of an in-country living collection of representative genotypes, with at least two duplications.</td>
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<td>Establishment of a cryopreserved seed bank, and associated research program to develop optimal long-term storage protocols, including synthetic seeds.</td>
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<tr>
<td>Establishment of <em>in vitro</em> germplasm collection, maximizing genetic diversity, and an associated research program to develop efficient protocols for <em>ex vitro</em> acclimatization for cultivation and population restoration.</td>
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<tr>
<td>Large-scale <em>in vitro</em> multiplication of certified planting material for prioritized genotypes.</td>
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<tr>
<td>Establishment of an orchid mycorrhizal and endophyte germplasm collection, and associated research into functional application of microbial symbionts in <em>ex situ</em> propagation, and population restoration &amp; cultivation.</td>
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<th>Circa situm conservation actions and sustainable use</th>
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<tr>
<td>Evaluation of market possibilities for diversified vanilla products from distinct species, and for organic or other product certification.</td>
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<tr>
<td>Establishment of community-based <em>circa situm</em> cultivation initiatives, including community germplasm banks as living collections.</td>
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<tr>
<td>Characterization of native <em>Vanilla</em> genetic resources for traits of agronomic and market interest, including phytosanitary evaluation of plant-microbe interactions.</td>
</tr>
<tr>
<td>Capacity building for good agro-ecological practice for vanilla cultivation, pollination and post-harvest processing.</td>
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</table>
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 habit. 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-Rodríguez 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 (Housholder 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 22°C. 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; Tanet 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-Alfar 2011, Porras-Alfar & 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 Basidiomycete 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, Khoiratty 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
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

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).
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.

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GENERA PLEUROTHALLIDINARUM: AN UPDATED PHYLOGENETIC OVERVIEW OF PLEUROTHALLIDINAE

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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 Pridegon, 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 undervided sister taxa of the other pleurothallid genera”. But, it was not until Pridegon and Chase...
(2001), based on the molecular studies by Pridgeon \textit{et al.} (2001), that \textit{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. Recircumscriptions 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 \textit{et al.} 2012, Karremans \textit{et al.} 2013a, 2013b, Wilson \textit{et al.} 2013, Karremans & Rincón-González 2015, Chiron \textit{et al.} 2016, Karremans \textit{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 \textit{Pleurothallis}, for example, are many genera that we know are not even closely related. The list includes \textit{Antilla} (Luer) Luer, \textit{Apoda-prorepentia} (Luer) Luer and \textit{Pleurobotryum Barb.Rodr.}, phylogenetically related to \textit{Acianthera}; it also lists \textit{Areldia} Luer, \textit{Cucumeria} Luer, \textit{Gerardoa} Luer and \textit{Rubellia} (Luer) Luer, which are actually \textit{Specklinia} Lindl. relatives; \textit{Andreettacea} Luer, with unknown affinity, and \textit{Sansonia} Chiron, basal in the pleurothallids, are also placed under \textit{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 \textit{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 $A_1 = B_1 + B_2$ is true. In contrast, in conflicting proposals that formula does not hold true. For example, the monophyletic genus \textit{Phloeophila} (sensu Pridgeon 2005) could be otherwise interpreted as the sum of the also monophyletic genera \textit{Luerella} Braas, \textit{Ophidion} Luer and \textit{Phloeophila} Hoehne & Schltr. These proposals are therefore alternative. However, the generic concept of \textit{Specklinia} (sensu Pridgeon 2005) is not equal to the sum of \textit{Acostaea} Schltr., \textit{Empusella} (Luer) Luer, \textit{Gerardoa}, \textit{Muscarella} Luer, \textit{Sarcinula} Luer, \textit{Specklinia} and \textit{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.


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.
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, Meyer & Cameron 2009, Karremans 2010, 2014, Endara 2011, Bogarin 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

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

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<th>Genus</th>
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<tr>
<td>Acianthera</td>
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<td>Muscarella</td>
<td>54</td>
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<td>Anathallis</td>
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<td>Myoxanthus</td>
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<td>Andinia</td>
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<td>Neocogniauxia</td>
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<tr>
<td>Andreettaea</td>
<td>1</td>
<td>Octomeria</td>
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<td>Atopoglossum</td>
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<td>110</td>
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<tr>
<td>Chamelophyton</td>
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<tr>
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<td>Pleurothallopsis</td>
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<td>Masdevallia</td>
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<tr>
<td>TOTAL</td>
<td>5,114</td>
<td></td>
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</table>

*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.
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.

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 2. Phylogenetic overview of the main affinities within the Pleurothallidinae based on the available DNA based studies.
proposed by Bogarin 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), Oxygia, 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 Oxygia 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 Cryptophoranthea erosus Garay, an unusual species known only from Hispaniola. It had been placed in Pleurothallis subgen. Acianthera sect. Cryptophoranthea 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. Cryptophoranthea (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 rhamical, and 2) an articulation between the pedicel and ovary, together with biological synapomorphies, including 3) auto-
incompatibility 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 *Fromiaria, 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 *Pannorphia* 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 *Lepanthespis*. Unpublished DNA data found *Expedicula* species embedded within the latter, it cannot therefore be recognized without rendering *Lepanthespis* polyphyletic. *Pannorphia*, 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 *Pannorphia, Anathallis sertularioideis* (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 leanthiform 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*.


**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 ramiacuals and tubular flowers with a synsepal and two pollinia, characteristics that are
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 aryer* Luer and *P. determammii* Luer; DNA data placed them within *Pabstiella* (Karremans *et al.* 2013a).


**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 Jazmin Alomía based on Bogarin 5512 (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., Crocodeilanthus (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 Condyloago 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 Condyloago, Crocodeilanthus, Dracontia, Effusiaella, Elongatia, Lomax, Niphatia, 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 Pleurothallids s.s. Their analyses also demonstrated that genera such as Effusiaella and Elongatia (Luer) Luer are polyphyletic and cannot be recognized as currently defined, and that genera such as Crocodeilanthus, Dracontia, Physosiphon, 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 Restrepi**

The eight genera accepted in this affinity -- Barbosella Schltr., Chamelophytont Garay, Dresslerella Luer, Echinosepala Pridgeon & M.W.Chase, Myoxanthus Poepp. & Endl. (Fig. 9), Pleurothallopsis Porto & Brade, Restrepiapull 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.
Affinity Specklinia

This affinity is currently made up of seven accepted genera: Andinia (Luer) Luer, Dryadella Luer, Muscarella Luer, Platystele Schltr. (Fig. 10), Scaphosepalum Pfizter (Fig. 11), Specclinia 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, Specclinia, 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 Specclinia was sister to a clade composed of Platystele and Scaphosepalum; sister to all of them was Dryadella. Luer (2002b) did not recognize Specclinia 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 Specclinia. A phylogenetic analysis including a broader sampling of Specclinia 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 Specclinia (Karremans 2015, Karremans et al., in press).

The new study also shows that Muscarella Luer should be excluded from Specclinia if it is to be maintained as monophyletic, because Muscarella is sister to the clade that includes Platystele, Scaphosepalum, and Specclinia. 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 Andinia (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 Specclinia 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


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. Specclinia because of the abbreviated ramiqual. Nevertheless, we know now that this feature has appeared many times independently in Pleurothallidinae, and the species then assigned to P. subgen. Specclinia 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 Specclinia. 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).

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 braslica* 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 braslica* 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 Zygozetalinae. 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.”

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LITERATURE CITED


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

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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

Prolongue. 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
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 annum, 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
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
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

<table>
<thead>
<tr>
<th>Country or Region</th>
<th>Estimated Number of Orchid Species</th>
<th>Land Area, 1000 km²</th>
<th>No. Species/1000 km²</th>
<th>Maximum Age of Elevation Start, Ma</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Guinea Island (Micro-Continent = West Papua + Papua New Guinea)</td>
<td>2759⁷ 3200²</td>
<td>786 (~235 in the Central Cordillera)</td>
<td>3.5 (11.7) 4.1 (13.6)</td>
<td>~12</td>
<td>Severely undersampled; 98% endemic!</td>
</tr>
<tr>
<td>Ecuador</td>
<td>4056⁴</td>
<td>248 (128 minus the Amazonia lowland)</td>
<td>16.4 (31.7)</td>
<td>&lt;15 acceleration at 4-6</td>
<td>~20% endemic³</td>
</tr>
<tr>
<td>Colombia</td>
<td>3960⁴ 4270⁵ 4354⁶</td>
<td>1197.4 (794 minus the Amazonia lowland)</td>
<td>3.3 (5.0) 3.6 (5.4) 3.6 (5.4)</td>
<td>4 and 15</td>
<td>Undersampled and underreported, ~37% endemic⁶.</td>
</tr>
<tr>
<td>Panama</td>
<td>1360⁷</td>
<td>74.3</td>
<td>18.3</td>
<td>4 to 15 (Seaway closure)</td>
<td>Under-sampled especially east of Canal Zone. 21.7% endemic⁷</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>1316⁸ 1461⁹ 1519¹⁰ 1598¹¹</td>
<td>51.1</td>
<td>25.7 28.6 29.7 31.3</td>
<td>0.5 to 7</td>
<td>Growth in species counts due to increased sampling effort and improved taxonomic methods. ~30% endemic¹²</td>
</tr>
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</table>

References Cited in this Table
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
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 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).
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...
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.
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 Guayaquil 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 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-González, 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

**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.
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), Ortiz Valdizieso 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.

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LITERATURE CITED


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

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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 parphyletic. 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 phylogenetic 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 species 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.

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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).
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* (Pansarin 2010b).


*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 & Markcs 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). *Cleistes* and *D. 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 & Markcs 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
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 *Pogoniae* 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 (Boyd 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.

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 sclerophyllym 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 Prigden 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. sclerophyllym.
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 *Epistephiium 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 *Epistephiium 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 *Epistephiium 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 *Epistephiium* 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.

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

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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,
incendios, animales, pérdida 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 micorrizica. 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).
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 et al. 2004, Otero et al. 2005, Otero et al. 2007, Pereira 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 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*.

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 de 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 H₂O 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 (H₀) es: si el glifosato afecta negativamente el crecimiento de hongos endófitos de *E. melinanthum*, y en segundo lugar, la H₀ 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 entre la hoja y el tallo 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).
Curvatura de hojas: luego de la aplicación del glifosato se observó 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 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.
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...
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 alejadas 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, 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**


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WHY WE HAVE NO SERIOUS ALTERNATIVES BUT COOPERATIVE TAXONOMY

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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
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 alphataxonomy, 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, photocopics, 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.

Figure 2. Lankester Composite Digital Plate of *Brassia* cf. *caudata*. Costa Rica, Caribbean morph, *D. Bogarin 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 folii radicalibus lanceolatis” (Plumier 1758), which McLeish and collaborators (1995) selected as the type for the species.
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 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.
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**


DETERMINANTS OF ORCHID SPECIES DIVERSITY IN LATIN AMERICA

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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 (Efímov 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
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.
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 \( S = a \cdot A \) + b and exponential for \( S = a \cdot \ln A \)) 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., \( \ln S = a \cdot A + b \cdot \ln A + c \).

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

<table>
<thead>
<tr>
<th>Model</th>
<th>Coefficient</th>
<th>Value</th>
<th>t</th>
<th>p</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S = a \cdot A + b \cdot \ln A + c )</td>
<td>a</td>
<td>0.0003</td>
<td>4.15</td>
<td>&lt; 0.05</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.11</td>
<td>1.64</td>
<td>0.12</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>4.41</td>
<td>5.84</td>
<td>&lt; 0.05</td>
<td>0.58</td>
</tr>
</tbody>
</table>

**Figure 1.** The species-area relationship, for temperate and tropical countries separately.
Discussion. The model $\ln S = a*\text{Alt} + b*\ln A + c$ 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.

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LITERATURE CITED


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

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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 (Dearmaley 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; Jersákova & 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...
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 the recent studies in fungi and whole genome studies of Agaricomycotina (Basidiomycota) we may now better understand why just these fungal groups became orchid mycobionts (Hibbet & Matheny 2007, 2014; Veldre et al. 2013). Tulansnelloid fungi are worldwide 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* (*Melastomataceae*) 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 (Tedesco 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. 2010). 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 Psygmonchis 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 Inundibura, 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; Dearmaley 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).

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