THE PLEUROTHALLIDINAE: EXTREMELY HIGH SPECIATION DRIVEN BY POLLINATOR ADAPTATION

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The generic and subgeneric classification of Pleurothallidinae has traditionally been a hazardous task. The main challenge has been understanding the underlying relationships of the immensely diverse, +5100 accepted species in the subtribe. Species groups that could be easily separated from others by means of specific floral traits have been shown to be non-monophyletic using molecular techniques. Morphological characters related to pollination that have frequently been used to group species, such as anther position and pollinia morphology, have evolved independently in most of the major clades of Pleurothallidinae. Adaptation to specific pollinators is likely to be one of the main drivers for morphological similarity in the reproductive organs of unrelated species. Myophily, or pollination by flies, may be common to members of the subtribe; however, the pollinators of most species and species groups are still unknown. We have compiled a dataset of pleurothallid pollinators by combining pollination reports from the literature and additional unpublished observations and have plotted the occurrence of diverse Diptera families across the Pleurothallidinae phylogeny. As far as we can tell, floral visitors have been documented for only about one fourth of the genera (i.e., *Acianthera, Andinia, Dracula, Lepanthes, Echinosepala, Masdevallia, Octomeria, Phloeophila, Pleurothallis, Porroglossum, Specklinia, Stelis, Trichosalpinx*, and *Teagueia*), and just about 2% of all known species belonging to the subtribe. Many of these reports are made for the first time, and most are based on few observations. The number species and genera of Pleurothallidinae for which pollination data are available is far from being enough to allow for an accurate estimation of all the different orchid-insect interactions. A robust DNA-based phylogeny of the subtribe, however, allows adequate placement of known relationships. Diverse pollination systems employing flies of the families Anthomyiidae, Calliphoridae, Cecidomyiidae, Ceratopogonidae, Chloropidae, Drosophilidae, Keroplatidae, Mycetophilidae, Otitidae, Phoridae, Richardidae, Sarcophagidae, Sciaridae, Tephritidae, and Ulidiidae are found in the subtribe. Most of them are shown to have evolved several times independently, and adaptation to one family or another occurs even among closely related species. Finally, based on the young age of the subtribe in contrast to that of these Diptera families, pollination systems in the Pleurothallidinae are most likely due to the orchid adapting to a preexisting insect/behavior rather than a case of coevolution.

Pollinator adaptation may be the single most important driving force of the remarkable floral diversification in orchids. Jersáková *et al.* (2006) argued that this adaptation is probably unilateral, without change in the pollinator (Williams 1982), and co-evolution between orchids and their pollinators is apparently uncommon (Szentesi 2002). Orchids frequently exploit existing plant–pollinator relationships or even sexual systems of insects, exemplified by species that achieve pollination through deception, not offering floral rewards (Ackerman 1986; Jersáková *et al.* 2006; Ramírez *et al.* 2011).

Pollination by deceit is well known among orchids and has been frequently considered another key innovation contributing to the high species richness of the family (van der Pijl and Dodson 1966; Cozzolino and Widmer 2005). Food deception has evolved repeatedly in different angiosperm groups but is mostly restricted to a few species per family (Renner 2005), whereas estimates indicate that a third of all orchids might be food-deceptive (Ackerman 1986), and it seems to have arisen many times independently in Orchidaceae. Sexual deception has been reported in several phylogenetically unrelated orchid clades (van der Pijl and Dodson 1966; Adams and Lawson 1993; Singer 2002; Ayasse *et al.* 2003; Singer *et al.* 2004; Blanco and Barboza 2005; Ciotek *et al.* 2006; Phillips *et al.* 2009; Peakall *et al.* 2010). Nectaries, nectar guides, and osmophores, required in reward- and deception-syndromes, are well known in the flowers of Pleurothallidinae (Vogel 1990; van der Cingel 2001).

Pleurothallidinae today include >5100 species (Karremans 2016) and continue to grow at a steady rate of about 85-90 species per year (Karremans and Davin 2017), making it probably the most species-rich subtribe in orchids and one of the largest among flowering plants. The generic and subgeneric classification of Pleurothallidinae has traditionally been a hazardous task, and understanding the underlying relationships of the species in the subtribe is one of the main challenges. Species groups that could be easily separated from others by means of specific floral traits have been proven non-monophyletic using molecular techniques (Pridgeon *et al.* 2001). Morphological characters related to pollination, such as lip features, anther and stigma position, and pollinia morphology are found to have evolved independently in most of the major clades of Pleurothallidinae.

One of the factors allowing such great diversity and causing these convergences in reproductive organs is undoubtedly adaptation to fly pollination. Myophily is currently considered the second most common pollination syndrome in Orchidaceae, with an estimated 15–25 % of the whole family being pollinated by flies (Christensen 1994; van der Pijl and Dodson 1966; Borba and Semir 2001). Most thorough pollination studies in the subtribe report fly pollination, including in the genera *Acianthera* Scheidw. (Borba and Semir 2001; de Melo *et al.* 2010; Pansarin *et al.* 2016), *Dracula* Luer (Endara *et al.* 2010; Policha *et al.* 2016), *Lepanthes* Sw. (Blanco and Barboza 2005), *Octomeria* R.Br. (Barbosa *et al.* 2009), *Pleurothallis* R.Br. (Duque-Buitrago *et al.* 2014), *Specklinia* Lindl. (Karremans *et al.* 2015a), *Stelis* Sw. (Albores and Sosa 2006), and *Trichosalpinx* Luer (Bogarín *et al.* 2018). Pleurothallidinae is therefore the largest fly-pollinated group in Orchidaceae. Few pollination syndromes in Pleurothallidinae have been studied in depth, and many additional, sometimes unsubstantiated, observations are found in the literature. Numerous authors have reported hummingbirds and insect orders including Coleoptera, Hemiptera, Hymenoptera, Lepidoptera, and Tysanoptera as possible pollinators of Pleurothallidinae species (van der Pijl and Dodson 1966; Dod 1986; Duque 1993). Even though these representatives of these groups may be found on flowers, in search of nectar for example, most of them are unlikely to be actual pollinators, as described further on. At this time it can be said that in this great diversity of Pleurothallidinae species there are surely numerous different pollination strategies employing a large number of pollinator species and species groups. Most of these syndromes still need to be discovered, but what is known today is described here (Table 1).

'able 1. Recorded Diptera species, genera and families visiting species of Pleurothallidinae.

Pleurothallidinae		Visiting Diptera		Pollinia Removal	Reference
Genus	Species	Species	Family		
Acianthera	*aberrans*		Phoridae	Not observed	Unpublished
Acianthera	*adamantiensis*	*Apallates nigricoxa*	Chloropidae	Observed	Borba *et al.* 2001
Acianthera	*adamantiensis*	*Apallates* sp.	Chloropidae	Observed	Borba *et al.* 2001
Acianthera	*adamantiensis*	*Hippelates* sp.	Chloropidae	Observed	Borba *et al.* 2001
Acianthera	*adamantiensis*	*Trigonama* sp.	Chloropidae	Observed	Borba *et al.* 2001
Acianthera	*aphthosa*	*Acrosticta* sp.	Ulidiidae	Observed	Pansarin *et al.* 2016
Acianthera	*aphthosa*		Otitidae	Observed	Ribeiro *et al.* 2006
Acianthera	*fabiobarrosii*	*Hippelates neoproboscideus*	Chloropidae	Not observed	Borba *et al.* 2001
Acianthera	*fabiobarrosii*	*Tricimba* sp.	Chloropidae	Observed	Borba *et al.* 2001
Acianthera	*hamosa*		Phoridae	Observed	de Melo 2008
Acianthera	*johannensis*	*Hippelates carrerai*	Chloropidae	Observed	Borba *et al.* 2001
Acianthera	*johannensis*	*Hippelates neoproboscideus*	Chloropidae	Observed	Borba *et al.* 2001
Acianthera	*johannensis*	*Tricimba* sp.	Chloropidae	Observed	Borba *et al.* 2001
Acianthera	*limae*		Phoridae	Observed	de Melo 2008
Acianthera	*luteola*	*Megaselia* sp.	Phoridae	Observed	Singer and Cocucci 1999
Acianthera	*modestissima*		Phoridae	Observed	de Melo 2008
Acianthera	*ochreata*	*Hippelates parvicalcar*	Chloropidae	Not observed	Borba *et al.* 2001
Acianthera	*ochreata*	*Megaselia* spp.	Phoridae	Observed	Borba *et al.* 2001
Acianthera	*prolifera*		Chloropidae	Observed	de Melo 2008
Acianthera	*teres*	*Hippelates neoproboscideus*	Chloropidae	Not observed	Borba *et al.* 2001
Acianthera	*teres*	*Megaselia* spp.	Phoridae	Observed	Borba *et al.* 2001
Andinia	*pendens*		Sciaridae	Not observed	Álvarez 2011

Pleurothallidinae		Visiting Diptera		Pollinia Removal	Reference
Genus	Species	Species	Family		
Dracula	*chestertonii*		Drosophilidae	Not Observed	unpublished
Dracula	*chimaera*		Drosophilidae	Not Observed	unpublished
Dracula	*chiroptera*		Drosophilidae	Observed	Policha 2014
Dracula	*erythrochaete*	*Drosophila sp.*	Drosophilidae	Not Observed	van der Pijl and Dodson 1966
Dracula	*erythrochaete*	*Zygothrica sp.*	Drosophilidae	Observed	unpublished
Dracula	*felix*	*Zygothrica sp.*	Drosophilidae	Observed	Endara *et al.* 2010
Dracula	*houtteana*		Drosophilidae	Not Observed	unpublished
Dracula	*lafleurii*	*Zygothrica spp.*	Drosophilidae	Observed	Endara *et al.* 2010
Dracula	*marsupialis*		Drosophilidae	Not Observed	Endara *et al.* 2009
Dracula	*morleyi*		Drosophilidae	Observed	Policha 2014
Dracula	*pubescens*		Drosophilidae	Observed	Policha 2014
Dracula	*sodiroi*		Drosophilidae	Observed	Policha 2014
Dracula	*radiosa*		Drosophilidae	Not Observed	Endara *et al.* 2009
Dracula	*trigonopetala*		Drosophilidae	Not Observed	unpublished
Dracula	*vampira*		Drosophilidae	Not Observed	unpublished
Dracula	*vinacea*		Drosophilidae	Observed	unpublished
Dracula	*wallisii*		Drosophilidae	Not Observed	Endara *et al.* 2009
Dresslerella	*pertusa*		Sciaridae	Not Observed	unpublished
Echinosepala	*aspasicensis*		Drosophilidae	Not Observed	unpublished
Lepanthes	*amplisepala*		Sciaridae	Not Observed	unpublished
Lepanthes	*glicensteinii*	*Bradysia floribunda*	Sciaridae	Observed	Blanco and Barboza 2005
Lepanthes	*yubarta*	*Bradysia sp.*	Sciaridae	Observed	Calderón-Sáenz 2012
Lepanthes			Sciaridae	Not Observed	Blanco and Vieira 2011
Lepanthes			Sciaridae	Not Observed	unpublished

Pleurothallidinae		Visiting Diptera		Pollinia Removal	Reference
Genus	**Species**	**Species**	**Family**		
Lepanthes			Sciaridae	Not Observed	unpublished
Masdevallia	*angulata*		Sarcophagidae	Not Observed	unpublished
Masdevallia	*asterotricha*		Drosophilidae	Not Observed	unpublished
Masdevallia	*bicolor*	*Drosophila sp.*	Drosophilidae	Observed	unpublished
Masdevallia	*bicolor*	*Drosophila aff. immigrans*	Drosophilidae	Observed	unpublished
Masdevallia	*calura*	*Drosophila sp.*	Drosophilidae	Not Observed	unpublished
Masdevallia	*calura*		Drosophilidae	Observed	Ziegler 2011
Masdevallia	*demissa*	*Drosophila sp.*	Drosophilidae	Observed	unpublished
Masdevallia	*floribunda*		Drosophilidae	Observed	Cuervo Martinez 2012
Masdevallia	*fonsecae*	*Drosophila hydei/ repleta*	Drosophilidae	Observed	unpublished
Masdevallia	*fractiflexa*		Calliphoridae	Not Observed	Dodson 1962
Masdevallia	*fulvescens*	*Drosophila sp.*	Drosophilidae	Not Observed	unpublished
Masdevallia	*ignea*	*Drosophila sp.*	Drosophilidae	Not Observed	Ziegler 2011
Masdevallia	*infracta*		Drosophilidae	Observed	Cuervo Martinez 2012
Masdevallia	*lata*	*Drosophila sp.*	Drosophilidae	Not Observed	unpublished
Masdevallia	*luerorum*	*Drosophila sp.*	Drosophilidae	Not Observed	unpublished
Masdevallia	*melanoxantha*	*Drosophila sp.*	Drosophilidae	Not Observed	unpublished
Masdevallia	*pachyura*		Drosophilidae	Observed	Ziegler 2011
Masdevallia	*peristeria*		Calliphoridae	Not Observed	Light 1998
Masdevallia	*phoenix*		Drosophilidae	Not Observed	unpublished
Masdevallia	*picturata*	*Drosophila sp.*	Drosophilidae	Not Observed	unpublished
Masdevallia	*regina*		Drosophilidae	Not Observed	Ziegler 2011
Masdevallia	*rolfeana*		Drosophilidae	Not Observed	unpublished
Masdevallia	*striatella*	*Drosophila sp.*	Drosophilidae	Not Observed	unpublished

Pleurothallidinae		Visiting Diptera		Pollinia Removal	Reference
Genus	Species	Species	Family		
Masdevallia	*utriculata*	*Drosophila sp.*	Drosophilidae	Not Observed	unpublished
Masdevallia	*veitchiana*		Drosophilidae	Not Observed	unpublished
Masdevallia	*veitchiana*		Drosophilidae	Not Observed	unpublished
Masdevallia	*zahlbruckneri*	*Drosophila sp.*	Drosophilidae	Not Observed	unpublished
Octomeria	*crassifolia*	*Bradysia spp.*	Sciaridae	Observed	Barbosa*et al.* 2011
Octomeria	*grandiflora*	*Pseudosciara sp.*	Sciaridae	Observed	Barbosa*et al.* 2011
Phloeophila	*pelecaniceps*	*Drosophila aff. Simulans*	Drosophilidae	Not Observed	unpublished
Pleurothallis	*chloroleuca*		Sciaridae	Not Observed	unpublished
Pleurothallis	*colossus*		Anthomyiidae	Observed	Calderón-Sáenz 2011
Pleurothallis	*colossus*		Calyptratae	Observed	unpublished
Pleurothallis	*cordata*		Phoridae	Observed	unpublished
Pleurothallis	*cordata*		Sciaridae	Observed	Dodson 1962
Pleurothallis	*dorotheae*		Drosophilidae	Observed	unpublished
Pleurothallis	*dunstervillei*		Sciaridae	Not Observed	Endara *et al.* 2009
Pleurothallis	*eumecocaulon*	*Laccodrosophila* sp.	Drosophilidae	Observed	Dodson 1965
Pleurothallis	*helleri*		Ceratopogonidae	Observed	unpublished
Pleurothallis	*marthae*	*Mycetophila sp*	Mycetophilidae	Observed	Duque-Buitrago*et al.* 2015
Pleurothallis	*marthae*	*Bradysia sp*	Sciaridae	Observed	Duque-Buitrago*et al.* 2015
Pleurothallis	*microcardia*		Mycetophilidae	Not Observed	unpublished
Pleurothallis	*microcardia*		Keroplatidae	Not Observed	unpublished
Pleurothallis	*millei*		Sciaridae	Observed	unpublished
Pleurothallis	*navisepala*		Tephritidae	Not Observed	Pupulin *et al.* 2017
Pleurothallis	*navisepala*		Sciaridae	Not Observed	unpublished

Pleurothallidinae		**Visiting Diptera**		**Pollinia Removal**	**Reference**
Genus	**Species**	**Species**	**Family**		
Pleurothallis	*phyllocardia*	*Laccodrosophila sp.*	Drosophilidae	Not Observed	unpublished
Pleurothallis	*phyllocardioides*		Richardidae	Not Observed	unpublished
Pleurothallis	*phyllocardioides*		Sciaridae	Observed	unpublished
Pleurothallis	*phymatodea*		Drosophilidae	Not Observed	unpublished
Pleurothallis	*rowleei*		Sciaridae	Observed	unpublished
Pleurothallis	*ruscifolia*	*Laccodrosophila* sp.	Drosophilidae	Not Observed	van der Pijl and Dodson 1966
Pleurothallis	*ruscifolia*		Sciaridae	Observed	unpublished
Pleurothallis	*xanthochlora*	*Drosophila sp.*	Drosophilidae	Not Observed	Dodson 1962
Pleurothallis			Sciaridae	Not Observed	unpublished
Porroglossum	*hystrix*		Drosophilidae	Not Observed	Zelenko 2014
Scaphosepalum	*beluosum*	*Zapriothrica spp.*	Drosophilidae	Not Observed	Endara 2013
Scaphosepalum	*decorum*		Drosophilidae	Not Observed	unpublished
Scaphosepalum	*digitale*	*Zapriothrica spp.*	Drosophilidae	Not Observed	Endara 2013
Scaphosepalum	*dodsonii*	*Zapriothrica spp.*	Drosophilidae	Not Observed	Endara 2013
Scaphosepalum	*ophidion*	*Zapriothrica spp.*	Drosophilidae	Not Observed	Endara 2013
Specklinia	*alajulensis*	*Drosophila sp.*	Drosophilidae	Not Observed	Karremans *et al.* 2015c
Specklinia	*calyptrostele*		Ceratopogonidae	Observed	unpublished
Specklinia	*dunstervillei*	*Drosophila spp.*	Drosophilidae	Observed	Karremans *et al.* 2015b
Specklinia	*endotrachys*	*Drosophila spp.*	Drosophilidae	Observed	Karremans *et al.* 2015a
Specklinia	*fulgens*	*Drosophila sp.*	Drosophilidae	Not Observed	unpublished
Specklinia	*pfavii*	*Drosophila spp.*	Drosophilidae	Observed	Karremans *et al.* 2015a
Specklinia	*remotiflora*	*Drosophila spp.*	Drosophilidae	Observed	Karremans *et al.* 2015a

Pleurothallidinae		Visiting Diptera		Pollinia Removal	Reference
Genus	Species	Species	Family		
Specklinia	*spectabilis*	*Drosophila spp.*	Drosophilidae	Observed	Karremans *et al.* 2015a
Specklinia	*vierlingii*		Ulidiidae	Not Observed	unpublished
Stelis	*aemula*	*Bradysia sp.*	Sciaridae	Observed	van der Pijl and Dodson 1966
Stelis	*argentata*		Sciaridae	Not Observed	Endara et al. 2009
Stelis	*immersa*	*Megaselia sp.*	Phoridae	Observed	Albores and Sosa 2006
Stelis	*lankesteri*		Sciaridae	Observed	unpublished
Stelis	*latisepala*		Sciaridae	Observed	unpublished
Stelis	*oblongifolia*		Sciaridae	Not Observed	Endara *et al.* 2009
Stelis	*parvula*		Cecidomyiidae	Observed	Bogarín *et al.* 2016
Stelis	*pilosa*	*Megaselia spp.*	Phoridae	Observed	unpublished
Stelis	*segoviensis*		Chloropidae	Observed	unpublished
Stelis	*vulcanica*		Sciaridae	Not Observed	Endara *et al.* 2009
Stelis			Sciaridae	Observed	unpublished
Stelis			Sciaridae	Observed	unpublished
Stelis			Cecidomyiidae	Observed	unpublished
Stelis			Sciaridae	Not Observed	unpublished
Teagueia			Drosophilidae	Not Observed	unpublished
Teagueia			Drosophilidae	Not Observed	unpublished
Teagueia	*teaguei*		Sciaridae	Not Observed	unpublished
Trichosalpinx	*blaisdellii*	*Forcipomyia sp.*	Ceratopogonidae	Observed	Bogarín *et al.* in press
Trichosalpinx	*reflexa*	*Forcipomyia sp.*	Ceratopogonidae	Observed	Bogarín *et al.* in press

The Pleurothallidinae and their pollinators

Acianthera Scheidw.

The genus *Acianthera* currently includes about 300 species that are distributed from Mexico to Argentina and Uruguay, through Central America and the Antilles, and is most species-rich in Brazil (Karremans *et al.* 2016a). Thorough pollination studies carried out in Brazil have reported three different families of Diptera as pollinators. *Acianthera adamantinensis* (Brade) F.Barros, *A. fabiobarrosii* (Borba & Semir) F.Barros & F.Pinheiro, *A. johannensis* (Barb.Rodr.) Pridgeon & M.W.Chase, *A. ochreata* (Lindl.) Pridgeon & M.W.Chase, *A. prolifera* (Herb. ex Lindl.) Pridgeon & M.W.Chase, and *A. teres* (Lindl.) Borba have been reported to be pollinated by diverse species belonging to the Chloropidae family (Borba and Semir 2001; de Melo 2008; de Melo *et al.* 2010). *Acianthera hamosa* (Barb.Rodr.) Pridgeon & M.W.Chase, *A. limae* (Porto & Brade) Pridgeon & M.W.Chase, *A. luteola* (Lindl.) Pridgeon & M.W.Chase, *A. modestissima* (Rchb.f. & Warm.) Pridgeon & M.W.Chase, *A. ochreata*, and *A. teres* were reported to be pollinated by diverse species of Phoridae (Singer and Cocucci 1999; Borba and Semir 2001; de Melo 2008; de Melo *et al.* 2010). In the meantime, flies of the families Otitidae (Ribeiro *et al.* 2006) and Ulidiidae (Pansarin *et al.* 2016) have been recorded as pollinators of *A. aphthosa* (Lindl.) Pridgeon & M.W.Chase. In Colombia, a photograph by Sebastian Vieira shows a species of Phoridae visiting the lip of *A. aberrans* (Luer) Pupulin & Bogarín (Fig. 1A).

The fleshy, gaping flowers of *Acianthera* are characterized by a fusion of the lateral sepals into a concave synsepal; the petals lanceolate and denticulate; the lip thick, oblong, bicallous, with lateral lobes below the middle and a pair of auricules at the base; the anther incumbent with a pair of pollinia with whale-tail-like caudicles (Karremans *et al.* 2016a). Initial attraction is attained through sweet or foul odors released from osmophores found on the sepals (de Melo *et al.* 2010; Pansarin *et al.* 2016). The flies are reported to walk from the sepals toward the lip following nectar guides; the movable lip tips over when the fly reaches a certain point, closing the gap between the lip and column. While backing out the insect removes the pollinia by first touching the viscarium-rich rostellar flap and then the pair of caudicles with the scutellum (Singer and Cocucci 1999; Borba and Semir 2001; de Melo *et al.* 2010; Pansarin *et al.* 2016).

Fig. 1. A. *Acianthera aberrans* with Phoridae; B. *Andinia pendens* with Sciaridae; C. *Dracula chestertonii* with Drosophilidae; D. *Dracula erythrochaete* with Drosophilidae; E. *Dracula houtteana* with Drosophilidae; F. *Dracula trigonopetala* with Drosophilidae; G. *Dracula vampira* with Drosophilidae; H. *Dracula vinacea* with Drosophilidae; I. *Dresslerella pertusa* with Sciaridae; J. *Echinosepala aspasicensis* with Drosophilidae; K. *Lepanthes* with Sciaridae; L. *Lepanthes* with Sciaridae. (Photos: S. Vieira (A, L); L. E. Álvarez (B); C. Mesa (C, E, G); A. P. Karremans (D, J); A. Kay (F); N. Gutierrez (H, K); D. Amaral, courtesy of Vitalmedic (I).

Andinia (Luer) Luer

As recently circumscribed by Wilson *et al.* (2017a), *Andinia* includes more than 70 species. They are endemic to the Andean countries of Colombia, Ecuador, Peru, and Bolivia and are mostly found at mid- to high elevations. Álvarez

Fig. 2. A. *Lepanthes* with Sciaridae; B. *Lepanthes amplisepala* with Sciardiae; C. *Masdevallia angulata* with Sarcophagidae; D. *Masdevallia asterotricha* with Drosophilidae; E. *Masdevallia bicolor* with Drosophilidae; F. *Masdevallia calura* with Drosophilidae; G. *Masdevallia demisa* with Drosophilidae carrying pollinia; H. *Masdevallia fonsecae* with Drosophilidae; I. *Masdevallia fulvescens* with Drosophilidae; J. *Masdevallia lata* with Drosophilidae; K. *Masdevallia luerorum* with Drosophilidae; L. *Masdevallia pachyura* with Drosophilidae. (Photos : F. Tovar (A, C); S. Moreno (B); C. Mesa (D); R. Vugt (E); C. Ziegler (F, L); C. M. Smith (G); D. Bogarín (H); A. P. Karremans (I, K); N. Cisneros (J))

(2011) published the only observation of pollination in this genus. The author showed *A. pendens* (Garay) Karremans & S.Vieira-Uribe being pollinated by Sciaridae in a manner that resembles pseudocopulation (Fig. 1B). The same syndrome has been described for *Lepanthes* Sw., a genus not closely related to *Andinia* (Karremans 2016), but in which many species of *Andinia* had been previously placed. The floral similarity of species of *Andinia* subgen. *Brachycladium* is likely a convergence in pollination strategy rather than true phylogenetic relatedness (Wilson *et al.* 2017a).

Dracula Luer

The mushroom like appearance and smell of *Dracula* flowers is well known among orchid scientists. Dentinger and Roy (2010) suggested that the lips mimic Agaricales mushrooms growing nearby. Endara *et al.* (2010), McNeil (2013), and Policha (2014) found that fungi-dwelling flies of Drosophilidae visit and pollinate *D. felix* (Luer) Luer and *D. lafleurii* Luer & Dalström. Diverse species belonging mostly to the genera *Zygothrica* and *Hirtodrosophila* (Drosophilidae) were the most commonly found visitors of those *Dracula* species (Policha 2014). The species of both are apparently known to use mushrooms at some stage in their life cycles. The *Dracula* species have been found to produce no nectar (Endara *et al.* 2010), instead mimicking the mushrooms visually and chemically (Policha *et al.* 2016). Yeasts, found growing on the flowers, may be actively involved in the pollination mechanism, especially the scent composition (McAlpine 2013).

Additional observations made on *D. chiroptera* Luer & Malo, *D. morleyi* Luer & Dalström, D. pubescens Luer & Dalström, and *D. sodiroi* (Schltr.) found that besides *Zygothrica* and *Hirtodrosophila*, species of the genera *Laccodrosophila* and *Zapriothrica* (Drosophilidae) also visited the flowers (Policha 2014). Endara *et al.* (2009) showed Drosophilidae on flowers of *Dracula marsupialis* Luer & Hirtz, *D. radiosa* (Rchb.f.) Luer, and *D. wallisii* (Rchb.f.) Luer. Flies of the same family are featured in photographs of *D. chestertonii* (Rchb.f.) Luer (Fig. 1C), *D. chimaera* (Rchb.f.) Luer, *D. erythrochaete* (Rchb.f.) Luer (Fig. 1D), *D. houtteana* (Rchb.f.) Luer (Fig. 1E), *D. trigonopetala* Gary Mey. & Baquero (Fig. 1F), *D. vampira* (Luer) Luer (Fig. 1G), and *D. vinacea* Luer & R.Escobar (Fig. 1H). It can be safely assumed that attracting Drosophilidae through mimicry of fungi is probably a widespread syndrome among *Dracula* species.

Dresslerella Luer

The genus *Dresslerella* includes 13 species (Karremans 2016) that are found growing from Guatemala to Peru (Pridgeon 2005). Their most distinctive features include the scurfy to pubescent leaves and a conspicuous connation of the dorsal sepal with with the synsepal, in some species leaving a small opening close to the apex for the pollinator to enter. Nothing is known about the pollination of *Dresslerella*, but based on the morphology of the lip, column, and pollinia the insect surely needs to step on the movable lip, where it is guided inward and may remove the pollinia while exiting backwards, as is observed in many other genera of Pleurothallidinae. A species of Sciaridae was photographed beside the flower of *D. pertusa* (Dressler) Luer (Fig. 1I) by Diego Amaral at Finca *Dracula* in Panama, but removal of pollinia was not observed.

Echinosepala Pridgeon & M.W.Chase

Not much is known about the pollination of the dozen or so species that belong to genus *Echinosepala*. Species of the genus have relatively large, solitary flowers that are borne either at the apex or basal nodes of the ramicauls (Pupulin *et al.* 2017a). The flowers are typically dark-colored, fleshy, with diversely hirsute and verrucose sepals and a lip with a prominent basal callus or pseudo-glenion containing a gelatinous, non-viscous, shiny exudate that is spread through a longitudinal groove to the apex of the lip, where it apparently dries, becoming matted, in two to three days (Pupulin *et al.* 2017a). An unpublished video shows a species of *Drosophila* (Drosophilidae) interacting with the verrucae on the sepals and attempting (and failing) to collect the gelatinous substance on the lip of *E. aspasicensis* (Rchb.f.) Pridgeon & M.W.Chase (Fig. 1J). The two flies stayed on the flower inspecting it thoroughly for a few hours without removing the pollinia.

Lepanthes Sw.

Lepanthes flowers, which are mostly characterized by the bilaminate lip with a central appendix, exhibit a highly specialized pollination system involving sexual deception. Flowers are specifically pollinated by male fungus gnats of the genus *Bradysia* (Diptera: Sciaridae), probably attracted by a pheromone-mimicking strategy (Blanco and Barboza 2005). *Lepanthes* includes more than 1000 species (Karremans 2016), so it is difficult to assess if pseudocopulation by Sciaridae can be generalized for the whole genus. Nevertheless, species of *Lepanthes* have standard floral features, and those structures known to be essential in the pollination syndrome are present with variations in most of its species. Additional published observations, including that of *L. yubarta* E.Calderon (Calderón-Sáenz 2012) and an unidentified *Lepanthes* species (Blanco and Vieira 2011; Fig. 1L) are both consistent with the initial findings in *L. glicensteinii* Luer (Blanco and Barboza 2005). So, too, are the unpublished findings for other *Lepanthes* species from Costa Rica (Bogarín, pers. comm.) and the photographs presented here by Nicolás Gutierrez (Fig. 1K), Francisco Tobar (Fig. 2A), and Sebastian Moreno (Fig. 2B) from Colombia and Ecuador. Claims that *Lepanthes* species are pollinated by Ceratopogonidae (Archila 2001) are unsubstantiated.

Masdevallia Ruiz & Pav.

More than 600 species are known to belong to the charismatic genus *Masdevallia*. Species are distributed from Mexico through Central America to Bolivia and Brazil and are most diverse in the Andes of Colombia and Ecuador (Pridgeon 2005). The species belonging to the genus are mostly recognized by the large colorful flowers, with caudate sepals that are diversely fused, frequently forming a conspicuous tube. The callous petals and movable lip are relatively short, the column small but elongate, the anther incumbent, the stigma ventral, and the pollinia with a pair of caudicles. Some species belonging to the this genus have large, brightly colored flowers, which has led authors to believe they might be hummingbird-pollinated (Dodson 1962a; van der Pijl and Dodson 1966).

Hummingbirds, curious as they are, may indeed be attracted by the brightly colored flowers and may even occasionally explore flowers of some species with their beaks. Nevertheless, Nunes *et al.* (2015) have shown that in order for the dark-colored pollinia to be placed on the beaks of hummingbirds, flowers of species of *Elleanthus* C.Presl must necessarily combine an easily accessible but narrow floral tube, continuous nectar secretion, and pollinia with a sticky viscidium. Similar requirements have been found in other orchid genera pollinated by hummingbirds, including *Arpophyllum* Lex. (pers. observ.) and *Stenorrhynchos* Rich. ex Spreng. (Singer and Sazima 2000). None of these features is present in *Masdevallia* species: pollinia are bright yellow; sticky viscidium is absent; there is no obvious flow of nectar; and frontal access to the tube is frequently impeded, as in *M. ignea* Rchb.f. and *M. rosea* Lindl. In fact, as expected from the size of the bird, speed of movements, and strength of the beak, hummingbirds are known to damage the delicate flowers they visit (Nunes *et al.* 2015).

The morphology of the large-flowered, brightly colored *Masdevallia* species is in essence no different from that of other species of the genus. The column and lip form a cavity that allows for a small insect to wander around. The insect steps on the movable lip that is delicately hinged to the column foot, and as it moves inwards, kept straight by the petals, its weight is shifted. This allows the lip to move toward the column, pressing the insect against it. The insect removes the pollinia by exiting backwards, smearing the viscarium present on the rostellum on the scutellum before touching and removing the pollinia. Cuervo *et al.* (2012) studied the morphology and morphometry of two of these large-flowered species, *M. coccinea* Linden ex Lindl. and *M. ignea*. The authors rejected the theory of hummingbird pollination, confirming the absence of nectar and suggesting that flies of Drosophilidae are the most likely pollinators.

Not surprisingly, observations in diverse species of *Masdevallia* from several countries consistently show that they are visited and pollinated by diverse Diptera, including the large, brightly colored species. Different Drosophilidae have been documented visiting the flowers of *M. asterotricha* Königer (Fig. 2D), *M. bicolor* Poepp. & Endl. (Fig. 2E), *M. calura* Rchb.f. (Fig. 2F), *M. demissa* Rchb.f. (Fig. 2G), *M. floribunda* Lindl. (Cuervo *et al.* 2012), *M. fonsecae* Königer (Fig. 2H), *M. fulvescens* Rolfe (Fig. 2I), *M. ignea*, *M. infracta* Lindl. (Cuervo *et al.* 2012), *M. lata* Rchb.f (Fig. 2J), *M. luerorum* Bogarín, Oses & C.M.Sm. (Fig. 2K), *M. melanoxantha* Linden & Rchb.f., *M. pachyura* Rchb.f. (Fig. 2L), *M. phoenix* Luer (Fig. 3A), *M. picturata* Rchb.f. (Fig. 3B), *M. regina* Luer (Fig. 3C), *M. rolfeana* Kraenzl., *M. striatella* Rchb.f., *M. utriculata* Luer (Fig. 3D), *M. veitchiana* Rchb.f. (Fig. 3E, 3F), and *M. zahlbruckneri* Kraenzl. Species belonging to the families Calliphoridae and Sarcophagidae (Diptera: Oestroidea) have been recorded visiting the closely related *Masdevallia angulata* Rchb.f. (Fig. 2C), *M. fractiflexa* F.Lehm. & Kraenzl. (Dodson 1962b), and *M. peristeria* Rchb.f. (Light 1998), all characterized by emitting a putrid, instead of sweet, odor and having a warty lip.

Octomeria R.Br.

A single pollination study involving species of *Octomeria* has been published. Barbosa *et al.* (2009) found that *O. crassifolia* Lindl. and *O. grandiflora* Lindl. were pollinated by males and females of species of the genus *Bradysia* (Diptera: Sciaridae) and a species of *Pseudosciara* (Diptera: Sciaridae), respectively. The insects initially landed on the sepals and then moved toward the movable labellum, shifting it when moving inward between the column and lip. Pollinia were removed by the flies while exiting backwards with the scutellum.

Pleurothallis R.Br.

In its current circumscription (Pridgeon 2005) the broadly distributed genus *Pleurothallis* includes about 465 species (Karremans 2016). Species belonging to the genus are florally varied, but in general terms they can be recognized by the lateral sepals fused into a synsepal that is similar to the dorsal sepal, the petals that are generally shorter and much narrower than the sepals, a triangular lip with a basal glenion, a compact column with an apical and stigma, and pollinia with a circular viscidium, which, contrary to those of *Platystele* and *Stelis*, is not a liquid drop.

Of its species, only a detailed pollination study of *P. marthae* Luer & R.Escobar has been published (Duque-Buitrago *et al.* 2014). The authors found that the species is visited and pollinated by flies of the Mycetophilidae and Sciaridae and that this occurs nocturnally. Observations by Díaz-Morales and Karremans (unpubl.) show that *P. rowleei* (Fig. 4D) and *P. ruscifolia* (Jacq.) R.Br. (Fig. 4F) are also pollinated by Sciaridae and that these remove pollinaria while reaching for the glenion at the base of the lip. In fact, Mycetophilidae and Sciaridae have been documented to visit several other closely related *Pleurothallis* species, including *P. aves-seriales* Luer & R.Escobar (Duque 1993), *P. chloroleuca* Lindl., *P. cordata* (Ruiz & Pav.) Lindl. (Dodson 1962b, as "*P. aff. cardiothallus*"; Duque 1993, as *P. monocardia*), *P. dunstervillei* Foldats (Endara *et al.* 2009), *P. microcardia* Rchb.f. (Fig. 3J), *P. millei* Schltr. (Fig. 3L), *P. phyllocardioides* Schltr. (Fig. 4B), *P. secunda* Poepp. & Endl. (Duque 1993), and an undetermined species (Fig. 4G).

Other Diptera families have also been recorded visiting and pollinating species of *Pleurothallis*. Díaz-Morales and Karremans (unpubl.) documented the pollination of *Pleurothallis eumecocaulon* Schltr. (Fig. 3I) by a species of *Laccodrosophila* (Drosophilidae), which is consistent with what was reported for the same species by van der Pijl and Dodson (1966). Species of the family Anthomyiidae (Diptera: Calyptratae) were shown to visit and remove pollinia of *Pleurothallis* colossus Kraenzl. ex Kerch. by Calderón-Sáenz (2011), and consistent with that observation, another Calyptratae was recorded removing pollinia of that species of *Pleurothallis* independently by Francisco Tobar (Fig. 3G). Visits by other Diptera families include records of Phoridae removing pollinia of *P. cordata* (Fig. 3H), Keroplatidae on *P. microcardia*

Fig. 3. A. *Masdevallia phoenix* with Drosophilidae; B. *Masdevallia picturata* with Drosophilidae; C. *Masdevallia regina* with Drosophilidae; D. *Masdevallia utriculata* with Drosophilidae; E. *Masdevallia veitchiana* with Drosophilidae; F. *Masdevallia veitchiana* with Drosophilidae; G. *Pleurothallis colossus* with Calyptratae; H. *Pleurothallis cordata* with Phoridae; I. *Pleurothallis eumecocaulon* with Drosophilidae; J. *Pleurothallis microcardia* with Mycetophilidae; K. *Pleurothallis microcardia* with Keroplatidae; L. *Pleurothallis millei* with Sciaridae. (Photos: E. Moron de Abad (A); C. Mesa (B); C. Ziegler (C); A. P. Karremans (D); S. Dalström (E); S. Vieira (F); F. Tovar (G, L); C. Mesa (H); M. Díaz (I); L. E. Álvarez (J, K))

(Fig. 3K), Tephritidae on P. *navisepala* Pupulin, J.Aguilar & M.Díaz (Pupulin *et al.* 2017b), Drosophilidae on *P. dorotheae* Luer, *P. phyllocardia* Rchb.f. (Fig. 4A), *P. ruscifolia* (van der Pijl and Dodson 1966), *P. tetragona* Luer & R.Escobar (Duque 1993), and *P. xanthochlora* Rchb.f. (van der Pijl and Dodson 1966), and Richardiidae on *P. phyllocardioides* Schltr. (Fig. 4C).

It must be stressed that species of *Pleurothallis* seem to be regularly visited by several insects that are not necessarily their pollinators. At the Bosque de Paz private reserve in Costa Rica, the authors recorded insects of several orders visiting the flowers of *P. phyllocardioides* (Fig. 4B). Similarly, the flowers of *P. helleri* A.D.Hawkes attracted different insects belonging to the orders Coleoptera, Diptera, Hymenoptera, and Lepidoptera.

Fig. 4. A. *Pleurothallis phyllocardia* with Drosophilidae. B. *Pleurothallis phyllocardioides* with Sciaridae. C. *Pleurothallis phyllocardioides* with Richardidae. D. *Pleurothallis rowleei* with Sciaridae. E. *Pleurothallis phymathodea* con Drosophilidae. F. *Pleurothallis ruscifolia* with Sciaridae. G. *Pleurothallis* with Sciaridae. H. *Scaphosepalum belusom* with Drosophilidae. I. *Specklinia calyptrostele* with Ceratopogonidae. J. *Specklinia fulgens* with Drosophilidae. K. *Specklinia remotiflora* with Drosophilidae L. *Specklinia vierlingii* with Ulidiidae. (Photos: N. Belfort (A); M. Diaz (B, D, F); I. Chinchilla (C); F. Tobar (E); S. Vieira (G); A. Kay (H); A. P. Karremans (I, K); W. Driessen (J); G. Barboza (L))

Only species of the Ceratopogonidae (Diptera) removed pollinia. The *Drosophila* visiting the flowers of *P. phymatodea* Luer (Fig. 4E) is another good example. It was carrying pollinia that were most likely from a *Masdevallia* or *Specklinia* but surely not of this *Pleurothallis*, which has much smaller pollinia with a viscidium and can only be placed on the legs or head, not the scutellum. Hymenoptera and Lepidoptera are among the other orders recorded visiting diverse *Pleurothallis* species. It is likely that these come in search of nectar, occasionally removing pollinia in the process. Pollen removal by ants, butterflies, and wasps is discussed in the section about non-Dipteran pollinators further on.

It is noteworthy that, notwithstanding the diversity in visitors, the pollinia of *Pleurothallis* are placed either on the legs or on the head of their pollinators. Pollinia attached to the legs have been recorded in *Pleurothallis colossus*, *P. cordata*, *P. eumecocaulon*, *P. marthae* but not in other members of the subtribe, whereas pollinia placed on the head have been documented in *P. millei*, *P. phyllocardioides*, *P. rowleei*, and *P. ruscifolia*. The pollination strategy seems to be, like that of *Stelis*, that the flattish and spreading flower has a rounded glenion at the base of the lip, which the insect attempts to reach. Close to the glenion is a compact column with an apical anther and broad stigma. The pollinia are removed when the insect touches the drop-like viscidium placed just above the glenion while reaching for it head-on. This mechanism allows for the pollinator group to be less specific than in genera in which the insect of a certain size and shape has to fit between the column and lip and tilt or trigger it or where sexual deception is employed. It remains to be proven whether some *Pleurothallis* species are truly generalists or whether their multiple, simultaneous flowers are simply part of a strategy in which most pollinia and nectar are lost to inadequate insects but pollination is ensured by successful placement of pollinia on a few of the "right" insects.

Wilson *et al.* (2017b; 2017c) speculated that some *Pleurothallis* species may be pollinated through pseudocopulation based on particular features of the lip, however, these do not resemble those of other Pleurothallidinae genera, namely *Andinia* and *Lepanthes*, in which pseudocopulation has been proven.

Porroglossum Schltr.

Porroglossum includes about 52 species with an Andean distribution. There are no published studies detailing the pollination mechanism of this genus. Photographs by Henry Oakeley, one of which was published in Zelenko (2014), show flies of Drosophilidae on the flowers of *Porroglossum hystrix* Luer in Peru. Elsy Buitrago and Nicolás Peláez (pers. comm.) found flies of that same family pollinating *P. muscosum* (Rchb.f.) Schltr. in Colombia. Even though the details of how this pollination syndrome works are unclear, it is well established that the lip of *Porroglossum* is extremely sensitive to touch and triggers easily and swiftly. The trap lip likely catapults the insect toward the column, undoubtedly causing the pollinia to be placed by means of its sticky viscidium. The shape and orientation of the lip and column would indicate that the pollinia are placed on the rear of the insect. Such a mechanism is unlike any other in the subtribe.

Restrepia Kunth

The genus *Restrepia* includes about 55 species, distributed from Mexico to Peru and Venezuela with its center of distribution in the high Andes (Luer 1996). The species are mainly recognized by the ramicaul with imbricating, distichous, and laterally compressed sheaths. The dorsal sepal and the petals are clavate at the apex and have been suggested to function as osmophores (Vogel 1990). The lip is usually spotted or striped with a pair of narrowly uncinate processes on the hypochile (Luer 1996). Even though no pollinators have been observed, based on a micromorphological floral analysis Millner and Baldwin (2016) hypothesized that after being attracted by scents produced by the osmophores, a Dipteran pollinator lands on the synsepal and lip, and following tactile and olfactory signals walks onto the hypochile. There, the pollinators pass between the uncinate processes and under the column, where they can remove or deposit the pollinia. The authors also suggested that the distance between the processes is important for a precise fit of the pollinator. In Costa Rica, *Restrepia* flowers are approached by flies, but full documentation of their behavior has not been possible.

Scaphosepalum Pfitzer

Just over 50 species of *Scaphosepalum* are found growing from Costa Rica to Bolivia and the Guyanas, with a center of diversity in the Andean countries of Colombia and Ecuador. Species belonging to the genus are distinguished especially by the non-resupinate flowers and the lateral sepals forming a basally concave synsepal apically narrowed and thickened, usually with thickened, cushion-like calli on the distal portion, which have been considered osmophores (Endara 2013; Karremans *et al.* 2016b). Diverse photographs show dipterans of Drosophilidae visiting flowers of genus *Scaphosepalum*. Endara (2013) referred to an unpublished study in which *Scaphosepalum* beluosum Luer, *S. digitale* Luer & Hirtz, *S. dodsonii* Luer, and *S. ophidion* Luer were visited and pollinated by *Zapriothrica* species (Drosophilidae); photographs of all are featured in Endara *et al.* (2009). The authors explained that "flies spend most of their visits lapping at the surface of the osmophores and sepals, or scraping the inner blade of the synsepals" and that the "pollinating agents transfer their weight from the osmophore or synsepal to the deflexed region of the lip, causing the lip to tilt and trap the pollinator against the column. The thorax and abdomen of the pollinator rest on the deflexed portion of the lip and form an open angle where the pollinia are inserted into the scutellum of the pollinator." A Drosophilidae species was also photographed by Andreas Kay on *S. decorum* Luer & R.Escobar (Fig. 4H).

Specklinia Lindl.

The genus *Specklinia* includes about 100 species widely distributed throughout the Neotropics. Species belonging to the genus are characterized by a ramicaul much shorter than the leaf; lateral sepals frequently connate; petals shorter, obtuse and entire; a linear, movable lip that is parallel to the column; and nude pollinia that lack caudicles and a viscidium (Karremans *et al.* 2016b). Chase (1985) reported species belonging to *Drosophila* (Drosophilidae) pollinating a species of the *S. endotrachys* (Rchb.f.) Pridgeon & M.W.Chase complex, and this was confirmed by Pupulin *et al.* (2012) and Karremans *et al.* (2015b) for other closely related members of the species complex. A study by Karremans *et al.* (2015a) found that indeed several species of *Drosophila*, mainly of the *D. hydei-repleta* complex, visited and pollinated *S. dunstervillei* Karremans, Pupulin & Gravend., *S. endotrachys*, *S. pfavii* (Rchb.f.) Pupulin & Karremans, *S. spectabilis* (Ames & C.Schweinf.) Pupulin & Karremans, and *S. remotiflora* Pupulin & Karremans (Fig. 4K). *Drosophila* were also seen visiting (although not removing) pollinia of *Specklinia alajuelensis* Pupulin & Karremans (Karremans *et al.* 2015c) and *S. fulgens* (Rchb.f.) Pridgeon & M.W.Chase (Fig. 4J), both of which also belong to *S.* subgen. *Specklinia*.

Members of the *S. endotrachys* complex attract pollinators using aggregation pheromones released from the sepals. The flies, both male and female, can linger for several hours at a time, inspecting the flower in search of the nectar drops produced on the warts. Behavior while on the flowers would indicate courtship; however, copulation has seldom been observed, and their main activity is sucking on the nectar-rich warts. The flies step on the lip in such a way that it tilts, pressing the insect against the column, the insect escapes by exiting backwards and removing the pollinia with the scutellum in the process (Karremans *et al.* 2015a).

There are no published studies on the pollination of any of the members of the other four subgenera of *Specklinia*, but unpublished observations indicate they are pollinated by different Diptera families. On one hand, Díaz-Morales and Karremans (unpubl.) found two species of Ceratopogonidae visiting and pollinating the white flowers of *S. calyptrostele* (Schltr.) Pridgeon & M.W.Chase (Fig. 4I), a member of *S.* subgen. *Hymenodanthae* (Barb.Rodr.) Karremans. A video by Gabriel Barboza shows a fly of the family Ulidiidae entertained by the dark purple lip of *S. vierlingii* Baumbach (Fig. 4L), a member of *S.* subgen. *Sarcinula* Karremans, without removing pollinia. The orange color and fruity smell of members of *S.* subgen. *Specklinia* contrast strongly with the foul-smelling, dark purple-stained flowers of *S.* subgen. *Sarcinula*, and it is not difficult to image they are pollinated by a different syndrome and even by different Dipteran families.

Stelis Sw.

In its broadest circumscription, *Stelis* includes more than 1000 species and is the most species-rich genus in Pleurothallidinae together with *Lepanthes*. The species included in this broad circumscription of the genus have a common origin as multiple phylogenetic studies have shown. Nevertheless, it is clear that several different pollination syndromes can be found in diverse clades within *Stelis s.l.*, and these have mostly likely resulted in particular floral shapes that superficially do not suggest affinity. To disentangle these different syndromes they will be discussed separately.

Stelis s.s., or the typical *Stelis* species, are characterized by flat, triangular flowers, with large sepals and small petals and lip, a reduced column with an apical anther and stigma, and pollinia with a drop-like viscidium. They include the vast majority of the species of the genus and are found commonly throughout the Neotropics (Karremans *et al.* 2013). Despite their numbers and presence, little is known about their pollination. Christensen (1992) studied the reproductive biology of *Stelis argentata* Lindl. and observed occasional visitation by flies but was unable to document any pollination events. In Mexico, Albores-Ortiz and Sosa (2006) studied *Stelis hymenantha* Schltr. without recording pollination. Endara *et al.* (2009) photographed Sciaridae visiting diverse species of *Stelis s.s.*, including *S. argentata*, *S. oblongifolia* Lindl., and *S. vulcanica* Schltr., while a Cecidomyiidae was photographed with pollinia of *S. cf. parvula* Lindl. by Bogarín *et al.* (2016). Several additional independent observations by Diego Bogarín (Fig. 5G), Luis Eduardo Álvarez (Fig. 5C, 5E, 5H), and the authors (Fig. 5A, 5B, 5D) consistently found Cecidomyiidae and Sciaridae visiting and pollinating diverse species of *Stelis* s.s. The large open sepals serve as a landing platform from which the flies explore the flowers, finally directing their attention to the middle. The petals and lip are similar in shape and size to each other, and this pseudo-radial symmetry allows for complete access to the base of the lip. It is most likely the glenion that these insects attempt to access, head first. The compact column allows the insect to access the base of the lip with ease, forcing it to touch the sticky, drop-like viscidium, thus attaching the pollinia close to the mouthparts. The apical, broad, and commonly bilobed stigma allows for pollinia deposition from many angles. This is consistent with van der Pijl and Dodson's (1966) observation that a species of *Bradysia* (Sciaridae) got the pollinia attached to its mouthparts while moving from flower to flower and taking nectar from the lip of *S. aemula* Schltr. Calcium oxalate crystals are found on the lip and petals of many *Stelis* species, and even though we have not observed pollinators directly interacting with these, Chase and Peacor (1987) suspected that they could play a role in pollination.

Fig. 5. A. *Stelis* with Sciaridae; B. *Stelis* with Sciaridae; C. *Stelis* with Sciaridae; D. *Stelis* with Sciaridae; E. *Stelis* with *Cecidomyiidae; F. Stelis galeata with Drosophilidae*; G. *Stelis lankesteri* with Sciaridae; H. *Stelis latisepala* with Sciaridae; I. *Stelis pilosa* with Phoridae; J. *Teagueia* with Drosophilidae; K. *Teagueia* with Drosophilidae; L. *Teagueia* with Sciaridae. (Photos: M. Díaz (A, B, I); L.E. Álvarez (C, E, H); A. P. Karremans (D); K. Gil (F); D. Bogarín (G); A. Kay (J, K); A. Hirtz (L))

Effusiella Luer and *Unciferia* (Luer) Luer, considered synonyms of *Stelis* in the broad sense, belong to a clade that is sister to the clade that includes *Stelis* in the strict sense. They consist of a few dozen species which florally are completely different from *Stelis* s.s. The flowers are gaping, with the two lateral sepals completely fused into a synsepal. The column and lip are elongate and parallel to each other. The anther is incumbent, the stigma ventral, and the pollinia lacking a viscidium (Karremans *et al.* 2013). This floral morphology in general terms is similar to that found in *Acianthera*, *Pabstiella* Brieger & Senghas, and *Specklinia*, which explains why many authors and enthusiasts are so reluctant to accept their current placement. Nevertheless, they are phylogenetically closer to *Stelis* s.s., and their floral affinities may simply be due to convergence. Albores-Ortiz and Sosa (2006) found that *S. immersa* (Linden & Rchb.f.) Pridgeon & M.W.Chase is visited and pollinated by a species of the genus *Megaselia* (Phoridae). Species of the same phorid genus were found pollinating the sister species, *S. pilosa* Pridgeon & M.W.Chase (Fig. 5I; Díaz-Morales and Karremans, unpubl.). The authors also found that the closely related *S. segoviensis* (Rchb.f.) Pridgeon & M.W.Chase is pollinated by a species of the family Chloropidae. These are the same families that have been documented pollinating several species of the florally similar but phylogenetically distant members of *Acianthera*.

Crocodeilanthe Rchb.f., another synonym of *Stelis* in the broad sense, forms a monophyletic group that is the sister clade of *Stelis* in the strict sense. They are unlike *Stelis* s.s. in that the two lateral sepals form a synsepal, instead of them being free. The sepals are not equal to each other, and the petals are relatively elongate. However, they do resemble their sisters in the relatively short column and lip, the apical anther, and the pol-

Fig. 6. A. *Masdevallia* with Curculionidae. B. *Dracula* with Chrysomelidae. C. *Pleurothallis* with an aphid (Hemiptera) and ant (Hymenoptera). D. *Masdevallia campyloglossa* with Vespidae (Hymenoptera). E. *Pleurothallis canaligera* with Vespidae (Hymenoptera). F. *Pleurothallis divaricans* with Vespidae (Hymenoptera). G. *Stelis gelida* with Vespidae (Hymenoptera). H. *Pleurothallis* with Formicidae (Hymenoptera). I. *Pleurothallis quadrifida* with Ichneumonidae (Hymenoptera). J. *Stelis* with Figitidae (Hymenoptera). K. *Stelis* acuminata with Hymenoptera. L. *Pleurothallis cordata* with Lepidoptera. (Photos: A. Kay (A, B, C); N. Gutierrez (D); S. Vieira (E); E. Moron de Abad (F, L); L. E. Álvarez (G, K); D. Garcia (H); A. P. Karremans (I); C. Mesa (J))

linia with a viscidium. Their morphology is intermediate between a *Stelis s.s.* and *Stelis s.l.* Duque (1993) showed Drosophilidae with the pollinia of three species of *Crocodeilanthe* (as *Pleurothallis floribunda, P. galeata,* and *P. pluricaremosa*); in all three species the pollinia were placed on top of the head, indeed an intermediate between the placement near the mouthparts in *Stelis* s.s. and on the scutellum in *Stelis s.l.* Consistent with those observations, a photograph by Karen Gil in Colombia show another Drosophilidae carrying the pollinia of *S. galeata* (Lindl.) Pridgeon & M.W.Chase on the head (Fig. 5F).

Teagueia (Luer) Luer

Not much is known about the pollination biology of the 14 species currently belonging to this Andean endemic genus. The flowers are distinguished by having an ovoid to suborbicular lip with a deeply cleft disc, a short column with an apical anther and stigma, and pollinia with a drop-like viscidium. Photographs by Andreas Kay show Drosophilidae on diverse species of *Teagueia* (Fig. 5J, 5K), and a photograph by Alexander Hirtz shows what appears to be a fly of Sciaridae visiting *T. teaguei* (Luer) Luer (Fig. 5L). Neither was seen removing pollinia.

Trichosalpinx Luer

Trichosalpinx (sensu Bogarín *et al.* 2018) includes some 17 species distributed from Mexico, through Central America and the Antilles, to Bolivia and Brazil. Until recently there were no observations about pollination. Bogarín *et al.* (2018) found that *T. blaisdellii* (S.Watson) Luer and *T. reflexa* Mel. Fernández & Bogarín are pollinated exclusively by females of the genus *Forcipomyia* (Diptera: Ceratopogonidae). The studied species have purple flowers with a ciliate, papillose, and extremely sensitive lip that is attached to the column foot by a thin ligament. The papillose surface of the lip blade secretes proteins and carbohydrates that guide the insect inward. When the insect has reached a certain point, the lip tilts toward the column. Pollinia are removed when the insect exits backwards, smearing the the scutellum with the sticky viscarium present on the rostellum and then touching the caudicles on the pollinia.

The authors suggested that the presence of only females and secretion of protein rewards indicate that *Trichosalpinx* imitates a model aimed at stimulating the protein-collection behavior of females for egg production through a complex deceptive system likely related to kleptomyophyly (Bogarín *et al.* 2018). The overall similarity in micro- and macromorphological features of these *Trichosalpinx* species with some species belonging to the genera *Anathallis* Barb.Rodr. and *Lankesteriana* Karremans indicates these may represent cases of parallelism toward a similar pollination syndrome, as explained by Bogarín *et al.* elsewhere in these Proceedings.

Zootrophion Luer

The odd-looking flowers of species of *Zootrophion* are characterized by the fusion at the base and apex of the dorsal sepal with the fully fused lateral sepals, leaving a pair a window-like openings on each side of the flower. No pollinators of this genus have ever been documented; however, Darwin (1882) reported eggs of an insect near the base of the flowers of *Z. atropurpureum* (Lindl.) Luer (as *Masdevallia* fenestrata Lindl. ex Hook.) at Kew. About the species he wrote "the presence of these two minute windows shows how necessary it is that insects should visit the flower in this case as in that of most other orchids. How insects perform the act of fertilisation I have failed to understand. At the bottom of the roomy and dark chamber formed by the closed sepals, the minute column stands, and in front of it is the furrowed labellum, with a highly flexible hinge, and on each side the two upper petals; a little tube being thus formed. When therefore a minute insect enters, or which is less probable, a larger insect inserts its proboscis through either window, it has to find by the sense of touch the inner tube in order to reach the nectary at the base of the flower. Within the little tube, formed by the column, labellum, and lateral petals, a broad and hinged rostellum projects at right angles, which can easily be upturned. Its under surface is viscid". As inferred by Darwin, *Zootrophion* must be pollinated by a fly small enough to enter the cavity formed by the column and movable lip. Even though the flowers look radically different from other Pleurothallidinae, in essence the mechanism is not much different. The insect has to enter the cavity formed by the column and movable lip, and pollinia are probably removed when the insect exits backwards, smearing the mentioned viscid substance on the underside of the rostellum on its scutellum, after which it touches the caudicles and the pollinia are attached. The exact details of the whole syndrome, including which flies, why they enter these flowers, and how they reach the lip is still unknown. Bogarín *et al.* present further details on the floral anatomy of *Zootrophion* elsewhere in these Proceedings. It is noteworthy, nonetheless, that fly eggs were also reportedly found within flowers of *Z. endresianum* (Kraenzl.) Luer (Christensen 1994).

Pollen removal by non-Dipterans

Coleoptera

Weevils (Coleoptera: Curculionidae) were reported to visit species of *Pleurothallis* (Duque 1993) and even remove pollinia of a species of *Stelis* (Christensen 1994). A leaf beetle (Coleoptera: Chrysomelidae) was reported to visit *Pleurothallis* species and *Stelis gelida* (Lindl.) Pridgeon & M.W. Chase (Duque 1993). Both are commonly found visiting orchids, mostly damaging the flowers or stealing nectar (Fig. 6A, 6B) rather than pollinating.

Hemiptera

Aphids (Hemiptera: Aphididae) were reported by Dod (1986) as pollinators of small species of *Lepanthopsis* (Cogn.) Ames, *Lepanthes*, and *Pleurothallis*. This is highly unlikely (if not impossible) because aphids are far too small and have reduced movement. They would not be able to transport pollinia from one plant to another. They are, however, common pests that feed on plants and are frequently herded by ants for their sugary secretions (Fig. 6C).

Hymenoptera

Ants and wasps are probably the most common non-Dipteran visitors of Pleurothallidinae flowers. Both are regularly seen visiting orchids in search of nectar but are not commonly cited as pollinators.

Even though ants (Formicidae) have been cited as pollinators for orchids (Peakall 1989; Schiestl and Glaser 2012), in general terms they are not efficient pollinators as they are flightless. In fact, ants have only been proven to pollinate terrestrial orchids in windy, open fields in temperate regions, where plants are easier to access by walking, and a second plant to deposit the pollinia is reachable. For epiphytic orchids in the tropics this seems much less likely, as distances are greater and there is less impediment for flight. They do frequent Pleurothallidinae flowers as shown in a species of *Pleurothallis* (Fig. 6H) and have been cited as pollinators (Archila and Chiron 2015) but should not be regarded as such unless it is proven.Wasps (Vespidae) have been recorded visiting flowers of *Masdevallia campyloglossa* Rchb.f. (Fig. 6D), *Pleurothallis canaligera* Rchb.f. (Fig. 6E), *P. divaricans* Schltr. (Fig. 6F), and *Stelis gelida* (Fig. 6G) on occasion removing pollinaria with their mouthparts. It is possible that these wasps are only casual visitors in search for nectar. Independent observations by Duque (1993) also showing wasps removing and carrying pollinia of P. *divaricans* may indicate that this is not casual at all.

A parasitoid wasp (Ichneumonidae) was recorded exploring and removing pollinia of the sweet-smelling, bright yellow-flowered *Pleurothallis quadrifida* (Lex.) Lindl. (Fig. 6I). Another parasitoid wasp (Figitidae) was recorded stuck in the column/lip cavity of a *Stelis* species (Fig. 6J), while in a closely related species of *Stelis* another wasp was observed with pollinia on the scutellum (Fig. 6K). A parasitoid wasp (Braconidae) was also recorded removing pollinia of *Stelis sclerophylla* (Lindl.) Karremans (Duque 1993), which belongs to the same group as the latter two *Stelis* species.

Even though we prefer to remain conservative until a thorough pollination study involving Hymenoptera in Pleurothallidinae is carried out, the observations on some of these wasps do seem to indicate pollination. The overall similarity in shape and size of these wasps and the flies, together with the placement of the pollinia on the mouthparts and scutellum as frequently observed in dipterans, is indicative of an insect that is indeed suited for, rather than accidentally, removing pollinia.

Lepidoptera

A moth was reported by Christensen (1994) visiting a species of *Stelis*, and a butterfly has been photographed visiting a *Pleurothallis* (Fig. 6L). They are probably inspecting the flowers in the search for nectar; however, based on their shape and size, it is highly unlikely that they are true pollinators of any species of these genera.

Thysanopstera

Thrips were reported by Dod (1986) as pollinators of species of *Stelis*. Even though thrips have been reported to pollinate the plants they feed on, it is quite unlikely, because of their size, shape, and behavior, that they are involved in the pollination of Pleurothallidinae. They are pests of many plants, including orchids.

Conclusions

Pleurothallidinae are generally pollinated by Diptera and possibly Hymenoptera, and the syndromes found within the subtribe are diverse. Each genus and most likely many species groups within each genus employ particular strategies to ensure reproductive success. Closely related species may be pollinated through different strategies using flies of unrelated families, whereas distant relatives may use a similar strategy and share pollinators of the same family. The first may result in flowers of close relatives looking different from each other, whereas the second may result in non-related taxa having similar flowers.

Diverging floral morphology is especially noticeable in some broadly circumscribed genera, for example *Pleurothallis* and *Stelis*, which authors and enthusiasts alike still struggle to define and recognize. At the same time it seems difficult to convince some people that species with almost identical flowers, like some members of *Andinia* subgen. *Brachycladium* (Luer) Karremans & S.V. Uribe, *Lepanthes*, and *Salpistele* Dressler (= *Stelis* s.l.), are completely unrelated. The reason is that more emphasis has traditionally been given to overall floral appearance rather than other characters. Nevertheless, it is well established that floral features are under strong selective pressure from pollinators, and DNA evidence has been a powerful tool in helping to debunk many of these preconceptions. It is not our intention to suggest that floral morphology is not informative, quite the opposite. Most groups of closely related species do share many floral features that allow for their recognition; however, this does not mean that every species with a similar flower is a close relative, nor that different-looking ones are not closely related. Evidence for phylogenetic relatedness can come from many different morphological features; vegetative features, for example, have been given secondary importance, but species of *Stelis s.l.* are virtually identical without flowers, whereas *Andinia*, *Lepanthes*, and *Salpistele* are easily set aside when infertile. In essence, studies are best served when evidence from multiple sources is combined.

We are still far from having a comprehensive understanding of the diversity of pollination syndromes in the Pleurothallidinae. If the information available from thorough pollination studies is added to that of observed pollen removal, even those recording only visitation, we would still have information for less than 2% of all species belonging to the subtribe. In terms of genera the numbers are less dramatic, but still low. There is not a single published observation of visitation, let alone pollination, of any species of the genera *Anathallis*, *Andreettaea* Luer, *Atopoglossum* Luer, *Barbosella* Schltr., *Brachionidium* Lindl., *Chamelophyton* Garay, *Dilomilis* Raf., Diodonopsis Pridgeon & M.W.Chase, *Draconanthes* (Luer) Luer, *Dryadella* Luer, *Frondaria* Luer, *Gravendeelia* Bogarín & Karremans, *Lankesteriana*, *Lepanthopsis*

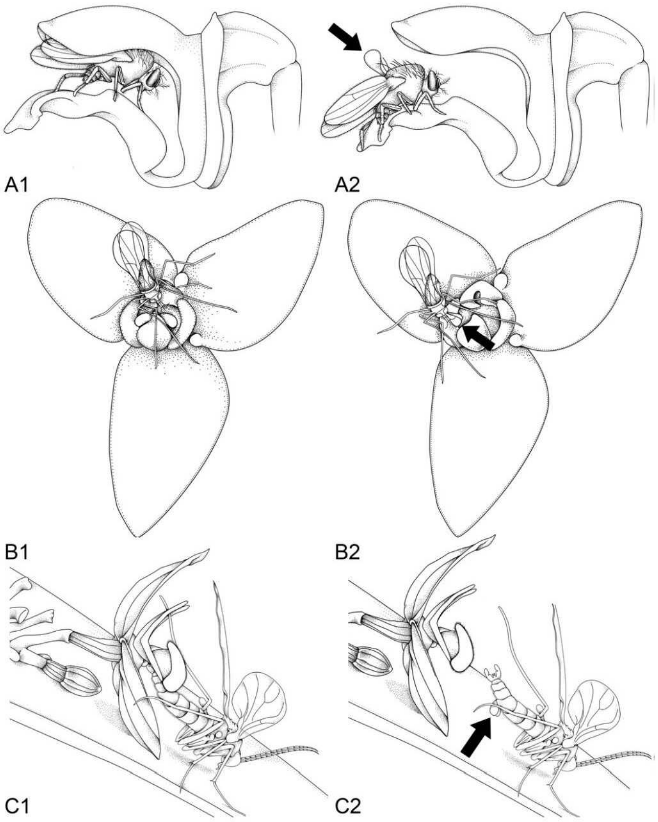

Fig. 7. General trends in the pollination mechanisms of Pleurothallidinae. A. Masdevalliform; B. Steliform; C. Lepanthiform. The arrows indicate pollinia placement. Illustrations by Lizbeth Oses.

(Cogn.) Ames, *Madisonia* Luer, *Muscarella* Luer, *Myoxanthus* Poepp. & Endl., *Neocogniauxia* Schltr., *Opilionanthe* Karremans & Bogarín, *Pabstiella*, *Pendusalpinx* Karremans & Mel.Fernández, *Phloeophila* Hoehne & Schltr., *Platystele* Schltr., *Pleurothallopsis* Porto & Brade, *Pesudolepanthes* (Luer) Archila, *Restrepia*, *Restrepiella* Garay & Dunst., *Sansonia* Chiron, *Stellamaris* Mel.Fernández & Bogarín, *Tomzanonia* Nir, *Trisetella* Luer, and *Tubella* (Luer) Archila. In addition the observations involving *Dresslerella*, *Echinosepala*, *Porroglossum*, *Teagueia*, and *Zootrophion* can hardly be considered more than records of insect visitation. This means that the pollination mechanisms have only been described for one fourth of the genera belonging to the subtribe.

Despite how little is known at this time, it is obvious that the diversity of strategies and the use of different pollinator groups in the subtribe are high. Flies belonging to many different Diptera families, including Anthomyiidae, Calliphoridae, Cecidomyiidae, Ceratopogonidae, Chloropidae, Drosophilidae, Keroplatidae, Mycetophilidae, Otitidae, Phoridae, Richardidae, Sarcophagidae, Sciaridae, Tephritidae, and Ulidiidae have been found visiting flowers of Pleurothallidinae, most of them removing pollinia. More importantly, similar pollination syndromes have been shown to have evolved several times independently, and adaptation to one family of flies or another occurs even among closely related species. Based on the young age of the subtribe (Pérez-Esobar and Chomicki *et al.* 2017) in contrast to that of the Diptera families (Wiegmann *et al.* 2011), orchids are most likely adapting to a preexisting insect/ behavior rather these relationships being a case of coevolution. However, a few general trends can also be pointed out.

On one hand, the mechanism of initial attraction through odors emitted from osmophores on the sepals, followed by a movement toward a movable lip using diverse guides and kept in place by the petals and column wings, the posterior tilting of the lip towards the column, smearing of the viscid substance of the rostellar flat on the scutellum, and subsequent removal of pollinia by touching the caudicles while exiting backwards from the column/lip cavity is a mechanism that can now be considered generalized in Pleurothallidinae. It has been described with variations in the genera *Acianthera*, *Dracula*, *Masdevallia*, *Octomeria*, *Scaphosepalum*, *Specklinia*, *Stelis* (*Effusiella* and *Unciferia*), and *Trichosalpinx*. The morphological features required for this mechanism to function are a combination of having a gaping flower, a movable lip with papillae that serve as guides, the lip and column being elongate and parallel to each other, an incumbent anther, a ventral stigma with a conspicuous rostellar flap with viscarium, pollinia flattened or with caudicles, and without a viscidium. Through this mechanism the pollinia are always placed, well centered, on the scutellum. Even though the details of the pollination syndrome of species belonging to *Echinosepala*, *Lankesteriana*, *Muscarella*, *Myoxanthus*, *Pabstiella*, *Pendusalpinx*, *Phloeophila*, *Tubella*, and *Zootrophion*, and others are unknown, based on their floral morphology a variation of the mechanism above is to be expected. This particular mechanism is here referred to as "masdevalliform" (Fig. 7A).

On the other hand, there is a mechanism involving flattish, spreading flowers with a rounded glenion at the base of the lip, provided with a compact column, in which the pollinia are removed when the insect touches the drop-like viscidium placed just above the glenion while reaching for it. Such a mechanism arose independently at least in the genera *Pleurothallis* and *Stelis*. It requires a glenion on the lip which the pollinators try to reach, a short column with an apical anther and stigma, and pollinia with a viscidium that is attached either on the head or legs. Based on the similarity in floral features, variations of this particular mechanism can be expected in *Brachionidium*, *Lepanthopsis*, *Platystele*, *Pseudolepanthes*, and *Teagueia*. This mechanism is referred to as "steliform" (Fig. 7B).

Finally, the striking similarity between flowers of *Andinia* and *Lepanthes* is associated with the use of a similar pollination syndrome. Species of both genera have been shown to be pollinated through a pseudocopulation strategy in which the flowers are probably emitting sexual pheromones, attracting male Sciaridae, which remove the pollinia while attempting to copulate with the flowers, engaging the midlobe of the lip, which has been termed the appendix. A bilobed lip with a central appendix and pollinia with a viscidium placed frontally on the column are also found in *Salpistele* (= *Stelis*) species, and it is likely that they, too, are pollinated by Sciaridae through pseudocopulation. From the fact that a sex pheromone is most likely employed in this syndrome, it is probable that the relationship between species of these genera and their pollinators is specific; however, this is not the case in other pleurothallids. This mechanism is referred to as "lepanthiform" (Fig. 7C).

Several species of *Pleurothallis* and *Stelis* attract many different insect groups to their flowers in what could be opportunistic pollination. Pollination through opportunism has been reported previously in Orchidaceae, and although not suggested before in Pleurothallidinae cannot be rejected a priori. Rewicz *et al.* (2017) found that *Epipactis helleborine* (L.) Crantz is visited and pollinated by Syrphidae, Cuclidae, Vespidae, Apidae, and Formicidae. Even though no studies definitively demonstrate that any Pleurothallidinae species is pollinated by insects from diverse orders, most studies including ample sampling of floral visitors and pollinators do show that two or more insect species are always involved. For *Specklinia*, diverse species mostly belong to a particular group within a single genus. In *Dracula*, species belong to different genera within a single family. In *Acianthera* and *Pleurothallis*, several species belong to different families within a single order.

A robust DNA-based phylogeny of the Pleurothallidinae is now available; however, the data that are available on pollination are far from sufficient to allow for an accurate estimation of all the different orchid-insect interactions to be expected. This hampers our understating of the frequency and influence of the evolutionary switches from one syndrome to another and their effect on speciation within the group. It is therefore of utmost importance that students and enthusiasts come forward with their observations on visitation and pollination of Pleurothallidinae, which by themselves may seem unimportant but when added up shed some light on the puzzle of evolution and complexity of the pleurothallids.

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