

Epidendrum (Orchidaceae) as a model system for ecological and evolutionary studies in the Neotropics

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Abstract The orchid genus *Epidendrum*, with 1500 species occurring within the Neotropical region, represents a very promising model system for evolutionary and ecological studies offering an expanded repertoire of research opportunities in the breadth of modern plant biology. *Epidendrum* displays a significant degree of morphological variation, chromosome number diversity and ecological interactions, which challenges widely held views on reproductive barriers and habitat selection. The widespread geographical distribution of many species and populations offers interesting opportunities to investigate how climatic changes and historic demographic processes shaped the current patterns of genetic and species diversity across different biomes and landscapes. Questions involving chromosome barriers to gene exchange and the role of postzygotic genetic barriers in species cohesion (e.g., the contributions of habitat selection and niche divergence on species cohesion) could be easily addressed when using the variety of natural hybrid zones found across *Epidendrum*. Several key evolutionary questions could be addressed with this model system, such as the identification of the first stages of adaptive radiation, the evolution of pollination strategies, the adaptive ecological significance of trait variation and hybridisation, the influence of historical demographic events on lineage diversification and speciation. With the advance of cost-effective molecular techniques and by combining ecological and phenotypic data, researchers can now tackle these questions and foster significant progress in the field of Neotropical plant diversification and evolution.

Keywords hybridization; Neotropical plant evolution; orchid pollination; orchid plant model; phylogeography; reproductive barriers; speciation

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■ INTRODUCTION

The Neotropical region harbors the greatest biodiversity on Earth, containing several of the world's biodiversity 'hotspots' (Myers & al., 2000). This region presents a complex biogeographic scenario and diversification processes on this continent cannot be restricted to a particular time interval or mechanism (Diniz-Filho & al., 2008; Antonelli & Sanmartín, 2011; Rull, 2011). The investigation of the origin of the huge biodiversity observed in the Neotropical regions is often limited by the paucity of available model organisms that could be used for addressing fundamental questions of evolutionary diversification processes in these regions.

Among tropical orchids, the genus *Epidendrum* L. is one of the largest plant genera in the Neotropics, with 1500 species occurring within the Neotropical region (from southeastern United States to northern Argentina). *Epidendrum* exhibits

remarkable morphological diversity and displays many interesting features related to ecological associations and geographic distribution (Hágsater & Soto-Arenas, 2005). It is the wealth of morphological variation, chromosome number diversity and ecological interactions that make *Epidendrum* a potential important model for studying a number of evolutionary questions, including habitat selection, reproductive biology, pollination mechanisms, speciation and reproductive isolation (Hágsater & Soto-Arenas, 2005; Pinheiro & al., 2010, 2011).

This new model system possesses interesting intrinsic features and offers great potential for investigating tropical orchid evolution in a way as evolution has been studied in temperate orchid genera, such as *Anacamptis* Rich. (Moccia & al., 2007), *Ophrys* L. (Xu & al., 2011) and *Dactylorhiza* Neck. ex Nevski (Paun & al., 2010). Unlike those orchids from temperate regions, several *Epidendrum* species are very easy to grow (Dunster-ville, 1979), can be easily bred as they flower nearly throughout

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the entire year (Pansarin & Amaral, 2008), can even be multiplied vegetatively by cuttings, and are thus convenient for experimental and field studies (Chen & al., 2002; Zettler & al., 2007), as has been illustrated with other proposed plant model systems from temperate regions (Wu & al., 2008; Bernasconi & al., 2009; Greimler & al., 2011). This partly overcomes the experimental problems commonly encountered in other long-generation orchid species that typically have a difficult germination stage and complex requirements for their cultivation.

Here, starting from the available knowledge about *Epidendrum* we aim to depict the state of scientific research in this genus, to identify and explore gaps of knowledge and to propose future directions, highlighting work in progress and new advances.

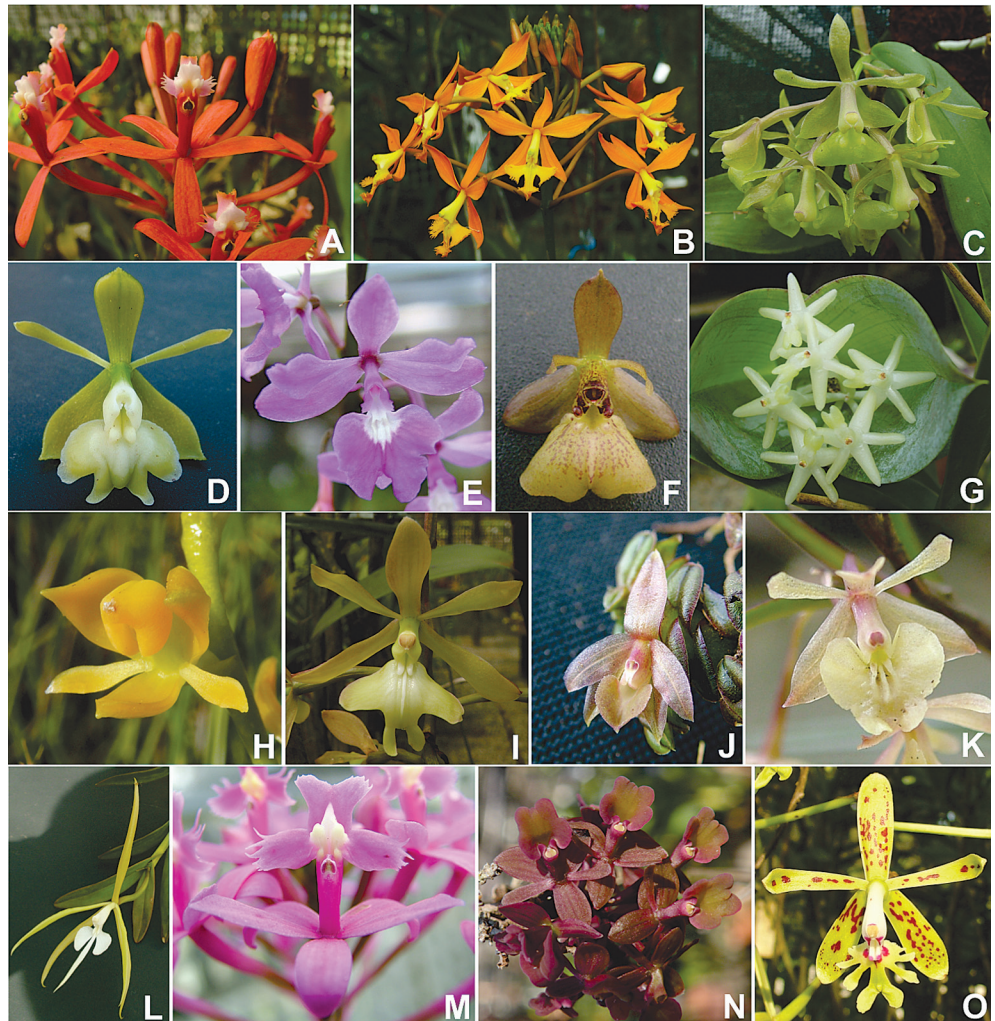
■ CIRCUMSCRIPTION AND DIVERSITY PATTERNS WITHIN EPIDENDRUM

The majority of the 1500 species are mainly found on the tropical forest slopes of the Andean and Guiana mountain ranges, including adjacent mountains in Central America

(Hágsater & Soto-Arenas, 2005). Remarkable phenotypic variation is observed across the genus (Fig. 1) which is mirrored within each species group (Brieger, 1978; Sastre, 1990a, b, 1991; Carnevali & Romero, 1996; García-Cruz & Hágsater, 1996; Pinheiro & Barros, 2007a, b) and even within some species (Electr. Suppl.: Fig. S1). As a consequence, the circumscription of *Epidendrum* and some related genera has been the subject of extensive debate (Dressler, 1967, 1984; Hágsater, 1984).

According to Hágsater & Soto-Arenas (2005), a few critical characters can be used to delineate the genus within its subtribe Laeliinae, such as the column united to the entire lip length, the rostellum parallel to the column axis and the nectary immersed within the pedicel (cuniculus). Hágsater & Soto-Arenas (2005) also expanded previous phylogenetic treatments of the genus (Van den Berg & al., 2000), including 80 species from different subgenera, accounting for most of the morphological variation observed in the group. The results confirmed previous taxonomic studies based on morphological characters (Lindley, 1852–1859; Brieger, 1977; Dressler, 1984; Hágsater, 1984). Central America and the South American Andean region appear to be the main centers of diversification for the genus.

Fig. 1. Morphological and colour variation of *Epidendrum* flowers of species from sections *Tuberculata* Lindl. (A), *Carinata* Lindl. (B, M), *Umbellata* Lindl. (C), *Polycladia* Lindl. (D), *Integra* Lindl. (E, F, N), *Equitantia* Lindl. (G), *Paniculata* Lindl. (H, I, O), *Spathaceae* Lindl. (J, L), *Holochila* Lindl. (K). **A**, *E. cochlidium* Lindl.; **B**, *E. incisum* Vell.; **C**, *E. proligerum* Barb. Rodr.; **D**, *E. densiflorum* Hook.; **E**, *E. campestre* Lindl.; **F**, *E. myrmecophorum* Barb. Rodr.; **G**, *E. vesicatum* Lindl.; **H**, *E. dendrobioides* Thunb.; **I**, *E. coronatum* Ruiz & Pav.; **J**, *E. schlechterianum* Ames; **K**, *E. filicaule* Lindl.; **L**, *E. nocturnum* Jacq.; **M**, *E. denticulatum* Barb. Rodr.; **N**, *E. anceps* Jacq.; **O**, *E. cristatum* Ruiz & Pav.



The ancestral species of several clades are confined to Mexico and Central America, suggesting that these regions may be the locations where early diversification of *Epidendrum* took place. A similar finding was observed also for other related genera of subtribe Laeliinae (Van den Berg & al., 2000, 2009).

Despite taxonomic and phylogenetic progress the question of why such species richness exists in *Epidendrum* has not been addressed, including whether some morphological novelties in the genus might represent key innovations that have triggered its amazing diversification. Similar questions have been investigated in some species-rich genera of other orchid groups thanks to newly available analytical methods, including the combination of statistical analyses of diversification rates with explicit phylogenetic hypotheses (Inda & al., 2012). A similar analysis of *Epidendrum*, even in the absence of significant representation in the fossil records, have become possible as a result of recently available calibration points, thus permitting age estimates for the primary lineages of *Epidendrum* (Gustafsson & al., 2010).

The species richness is clearly mirrored in the large array of different habitats occupied by *Epidendrum* species, which may reflect the diversity of the vegetative morphological features that are observed. The presence/absence of pseudobulbs, the variation in leaf number and thickness, and epiphyte/terrestrial/rupicolous habits are some examples of vegetative diversity found within the genus (reviewed by Hågsater & Soto-Arenas, 2005). Although terrestrial members are common, the vast majority of *Epidendrum* species are epiphytes, occurring in dry seasonal forests where they grow on Cactaceae and Velloziaceae species, or in humid rain forests on mountain slopes where light and moisture are better available in the canopy. This outstanding variation in life history and edaphic range makes *Epidendrum* a premier system for investigating the genetics of adaptive divergence and speciation (i.e., Pinheiro & al., 2009b, 2010, 2011) in a phylogenetic framework. In this regard, Pinheiro & al. (2009b) in a recent study investigated infrageneric relationships in *E.* subg. *Amphylottium* (Salisb.) Brieger, one of the most diverse groups of the genus. Their phylogenetic hypothesis reflects biogeographical and ecological affinities among species (Fig. 2), with clades composed of species mainly distributed along the Andean mountains (Andean clade) and the Brazilian coast (Atlantic clade), whereas species from the *E. secundum* complex were found in ruderal habits (*E.* sect. *Tuberculata* Lindl.).

The limited extent of genetic differentiation found among species (genetic distance = 0.01642–0.0004; Pinheiro & al., 2009b; Pessoa & al., 2012), phenotype–environment correlations (Hågsater & Soto-Arenas, 2005; Pinheiro & al., 2010) and fitness advantages of trait values in particular environments (Pinheiro & al., 2010) suggest a rapid adaptive radiation for this group. Multidisciplinary approaches are needed to test the hypothesis that *Epidendrum* represents an exemplar adaptive radiation (sensu Schluter, 2000). Particularly useful would be studies aiming to identify correlations between different phenotypes of descendant species and their divergent environments. Reciprocal transplant experiments and specific physiological traits related to different soil conditions (salinity, drought and

flooding tolerance) are fundamental subjects that need to be addressed in future studies. In this regard, *E.* subg. *Amphylottium* offers rich opportunities for identifying correlations between different genotypes and environment. In particular, species from the *E. secundum* complex could be very useful for such studies because most of these taxa have a weedy terrestrial habit and share the ability to grow in extreme and disturbed sites (Fig. 3)

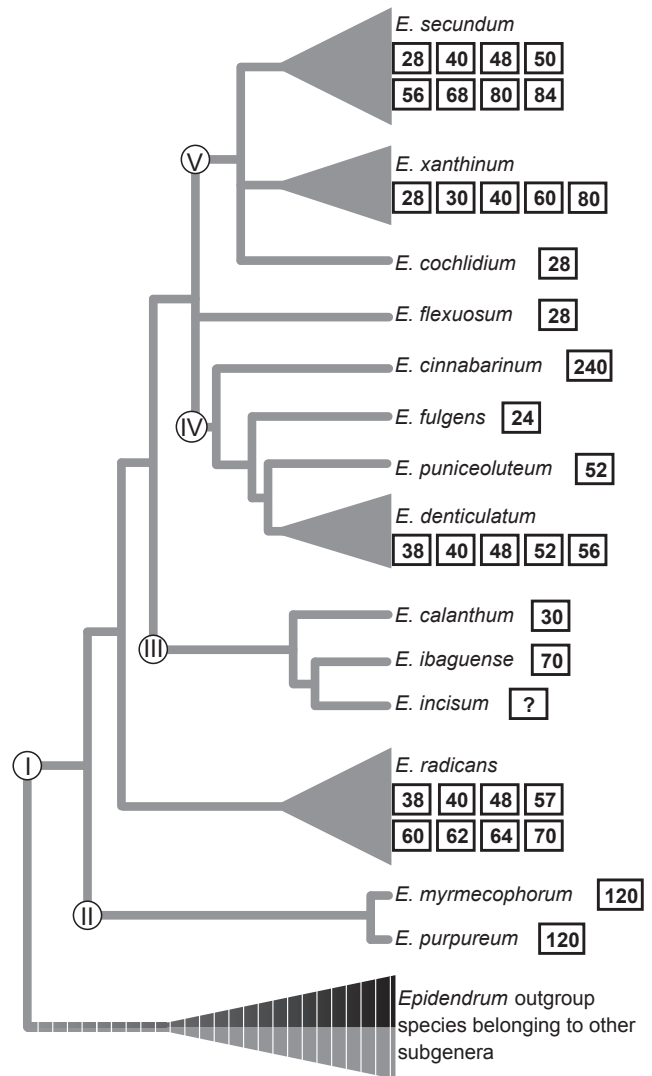


Fig. 2. Schematic representation of evolutionary relationships in *Epidendrum* subg. *Amphylottium* (I) and internal clades (II, *E.* sect. *Integra* Lindl.; III, Andean clade; IV, Atlantic clade; V, *E.* sect. *Tuberculata* Lindl.). Grey branches indicate clades in which free nectar is not observed in nectaries (cuniculus) and in which species are pollinated predominantly by butterflies. Diploid chromosome numbers are indicated inside squares. Outgroup species (striped black and grey) belonging to other subgenera (*E.* subg. *Aulizeum* Lindl., subg. *Spathium* Lindl., subg. *Euepidendrum* Lindl., subg. *Pleuranthium* Lindl., etc.) have a chromosome base number of $2n = 40$, and show several reproductive strategies ranging from complete outcrossing (with self-incompatibility) to autogamy (i.e., cleistogamy), nectar reward and deceptive pollination systems, and are pollinated by moths, butterflies or both. Modified from Pinheiro & al. (2009b).

such as roadside margins, cleared steep slopes after landslides, and sand dunes (Rosales & al., 1997; Hágsater & Soto-Arenas, 2005), and can easily be propagated vegetatively. Additionally, the large geographic distribution observed for this group, including different biomes in South and Central America (Hágsater & Soto-Arenas, 2005), offer an interesting opportunity to test adaptation related to different biotic and abiotic conditions, as has already been done for the temperate genus *Dactylorhiza* Neck. ex Nevski (Paun & al., 2010, 2011). Moreover, given that reproductive barriers are still incomplete within this group (Hágsater & Soto-Arenas, 2005; Pinheiro & al., 2009b, 2010; Pinheiro & al., in prep.), the first stages of adaptive radiation and speciation could be properly investigated in this species complex.

■ CHROMOSOME VARIABILITY AND PLOIDY LEVELS

Polyploidy is considered a prominent force in plant evolution and represents the most common mode of sympatric speciation in plants (Soltis & Soltis, 2009; Buggs & al., 2011). The fact that polyploids have an extra set of genes has been

proposed to result in various adaptive advantages, thus increasing the persistence of polyploid species (Fawcett & Van de Peer, 2010). Unreduced gamete formation may vary substantially across different populations, and environmental stress may contribute significantly to the establishment of polyploid species (Ramsey & Schemske, 1998).

Chromosome numbers are only known for a small fraction of *Epidendrum* (<3%). However, the 38 available counts are spread across the genus with $2n = 40$ as the most frequent count (Felix & Guerra, 2010). Nonetheless, despite the wide distribution of this base number, some small clades are known to exhibit extraordinary chromosome number variation. For instance, in section *Tuberculata* of the *Epidendrum* Atlantic clade, numbers range from $2n = 24$ to $2n = 240$, with several intermediate numbers found (as 28, 48, 52, 60 and 80) (Fig. 2). Closely related species and even adjacent populations also show large variation in chromosome number and ploidy, as has been observed in *Epidendrum secundum* Jacq., *E. radicans* Pav. ex Lindl. and *E. denticulatum* Barb. Rodr. (Assis, 2009; Pinheiro & al., 2009b; Felix & Guerra, 2010).

Polyploidisation events gave raise to high chromosome numbers in some *Epidendrum* species (Fig. 2), such as

Fig. 3. Extreme habitats occupied by *Epidendrum* species. **A**, Mountain slopes deforested after landslides; **B**, rocky mountain outcrops; **C**, roadside bank where *Epidendrum* species can be found; **D**, *E. warrasii* Pabst growing as an epiphyte attached to a rupicolous palm tree in dry outcrop vegetation; **E**, *E. fulgens* Brongn. growing on disturbed sand dunes on urban coastal beaches.



E. myrmecophorum with $2n = 120$ and *E. cinnabarinum* Salzm. ex Lindl. with $2n = 240$, which is the highest chromosome number known for orchids (Conceição & al., 2006; Pinheiro & al., 2009b). Interestingly, these species and others showing high chromosome numbers are found in disturbed and stressful habitats (Assis, 2009; Pinheiro & al., 2009b; Felix & Guerra, 2010). This is in accordance with theoretical studies which suggest that polyploids are more likely to evolve under harsh conditions (Ramsey & Schemske, 1998; Fawcett & Van de Peer, 2010). Identical chromosome numbers were found across different populations of *E. cinnabarinum*, indicating a stable ploidy level and suggesting that polyploidisation events took place in the early stages of species diversification (Felix & Guerra, 2010). Anaphase bridges and lagging chromosomes were observed in *E. cinnabarinum* meiotic cells, suggesting potential meiotic instability in this species. Nevertheless, only 8.2% of its pollen tetrads contained micronuclei, suggesting that meiotic instability has a limited effect on the fertility of *E. cinnabarinum* (Conceição & al., 2006).

To explore in more detail the role of ploidy variation in *Epidendrum* evolution, comparative studies should be planned with different species and hybrid zones. Surprisingly, genome size estimates are still lacking for this genus. In this regard, cytogenetic studies aiming to understand chromosomal variation at the population level are still needed, particularly in hybrid zones between parental species with different ploidy level/chromosome numbers. The use of flow cytometry can facilitate estimation of ploidy levels at the individual and population levels (Burton & Husband, 1999, 2000; Trávníček & al., 2011), which can otherwise be tedious and difficult via standard chromosome counting methods.

■ POLLINATION, REPRODUCTIVE ECOLOGY AND MATING SYSTEMS

Deceptive pollination, as food and sexual deception, has evolved repeatedly in different orchid lineages, and one-third of orchid species are estimated to deceive their pollinators (Cozzolino & Widmer, 2005). Both food-rewarding and food-deceptive species are found in *Epidendrum*, however, the relative proportion of rewarding versus deceptive species is unknown. Even when detailed studies are still missing, the typical attributes of food-deceptive orchids, such as infrequently visited flowers and low fruit set are commonly observed in *Epidendrum* species (Ackerman & Montalvo, 1990; Bush & al., 1999; Almeida & Figueiredo, 2003; Pansarin & Amaral, 2008; Fuhro & al., 2010, Pinheiro & al., 2010, 2011).

A diverse array of animals act as pollinators of *Epidendrum* species, but moth and butterfly syndromes are observed in almost all *Epidendrum* species. The keyhole flower structure, in which the insect introduces the proboscis within the cuniculus looking for nectar, is commonly observed in many species and was described in detail by Dressler (1981). Bright yellow and orange flowers are observed in butterfly-pollinated species, whereas white and pale green, strongly scented flowers are observed in moth-pollinated species (Van der Pijl & Dodson,

1966). Ornithophilous (*E. cinnabarinum*, *E. ibaguense* Humb. & al.) and myophilous (*E. medusa* Rchb. f., *E. fimbriatum* Kunth) species have also been observed (Hágsater & Soto-Arenas, 2005). As shifts from one pollination system to another could represent an important driving force for the evolutionary diversification of *Epidendrum*, the pollination systems should be mapped onto the phylogeny to analyse pathways of floral evolution and clade diversification, as has already been done in other orchid groups (*Disa*, Johnson & al., 1998; tribe Orchideae, Inda & al., 2012).

Nectar reward has been found in both butterfly- and moth-pollinated species (Adams & Goss, 1976; Braga, 1977; Goss, 1977; Pansarin, 2003). Nevertheless, large amounts of free nectar were observed in only a few species, including *E. vesicatum* Lindl. (Pinheiro, pers. comm.), *E. diffforme* Jacq. (Goss, 1977), *E. compressum* Griseb. and *E. huebneri* Schltr. (Braga, 1977). By contrast, the detection of nectar in the nectaries or cuniculus could be a difficult task for most species. Even when free nectar is not evident, some authors noted that nectar is released from thin-walled cells lining the cuniculus when the pollinator rasps them with fine spines (Adams & Goss, 1976; Pansarin, 2003). Rewarding and rewardless *Epidendrum* species are distinguished based only on the observation of free nectar availability in floral nectaries (Almeida & Figueiredo, 2003; Hágsater & Soto-Arenas, 2005; Pansarin & Amaral, 2008). Thus, detailed anatomical research aiming to detect the presence of secretory structures and nectar in *Epidendrum* flowers is needed to provide detailed data regarding the presence or absence of nectar and other pollinator reward compounds, such as floral oils and resins (Moreira & al., 2008). The occurrence in the same genus of both rewarding and deceptive species makes *Epidendrum* an ideal model system for the comparative study of the link between pollination efficiency and evolution of floral traits in rewarding and deceptive orchids in the context of a phylogenetic lineage.

Some nectarless *Epidendrum* species of subgenus *Amphylottium*, such as *E. radicans* (Bierzuchudek, 1981) and *E. fulgens* Brongn. (Fuhro & al., 2010), may strictly mimic some nectariferous species growing in their habitat (i.e., Batesian mimicry), primarily those of *Lantana* L. (Verbenaceae) and *Asclepias* L. (Asclepiadaceae). In these rewarding/deceptive mimetic systems, species occur in sympatry, flower simultaneously, share butterfly pollinators and have functionally related inflorescences and floral colours (Bierzuchudek, 1981; Fuhro & al., 2010). Long-term studies including data collected from different populations and transplant experiments, such as those performed by Johnson (1994) and Johnson & al. (2003) in South African orchids could be important for clarifying the role of Batesian mimicry in nectarless *Epidendrum* species.

Pollinator selection based on different flower scents can play an important role in orchid diversification (Mant & al., 2005; Xu & al., 2011), including species pollinated by Lepidoptera (Huber & al., 2005). Nevertheless, variation in floral scents was detected only among populations of *E. ciliare* (Moya & Ackerman, 1993), and thus the role of different floral scents in pollinator-mediated divergent selection is still an open question in *Epidendrum*. In this regard, the time is ripe for

combining gas chromatography and electrophysiological approaches already developed for the analysis of the scent of bee- and wasp-pollinated Mediterranean and Australian orchids (Schiestl & Marion-Poll, 2002; Peakall & al., 2010).

Food-deceptive orchids often show lower fitness (Scopece & al., 2010), and flower herbivory can contribute to this by severely limiting plant reproduction (Rico-Gray & Thien, 1989; Almeida & Figueiredo, 2003). Interestingly, many *Epidendrum* species that lack free nectar in the cuniculus have extrafloral nectaries. These structures feed ant species and keep them patrolling flowers, reducing the levels of herbivory (Delabie, 1995; Almeida & Figueiredo, 2003). Thus, reduced levels of nectar within flowers and the presence of extrafloral nectaries could represent a means to reduce herbivory without rising energetic costs, a hypothesis that needs to be tested in rewarding and non-rewarding *Epidendrum* species.

The genus *Epidendrum* also encompasses a wide repertoire of reproductive systems ranging from self-incompatibility to autogamy (Hágsater & Soto-Arenas, 2005). Catling (1990) reported autogamy for several species, and Iannotti & al. (1987) identified this mechanism in *E. rigidum* Jacq. Self-incompatibility has been reported for *E. anceps* Jacq. (Adams & Goss, 1976), *E. difforme* (Goss, 1977) and *E. densiflorum* Hook. (Pansarin, 2003). Pollen tubes in self-pollinated flowers of *E. densiflorum* showed irregular growth, with asymmetrical callose deposition along the pollen tube wall, close to the stigma (Pansarin, 2003). In self-compatible taxa, geitonogamy is restricted to nectarless species, which do not offer any reward to pollinators (Almeida & Figueiredo, 2003; Pansarin & Amaral, 2008; Fuhro & al., 2010). Deceived pollinators do not try to visit other flowers of the same inflorescence, and leave the patch after only a few attempts to collect nectar (Cozzolino & Widmer, 2005). Self-compatible but non-autogamous *Epidendrum* species vary in the amount and pattern of molecular genetic variation in a way that is consistent with their mating systems. Population surveys of microsatellite variation in food-deceptive species (Pinheiro & al., 2010, 2011) showed that the predominately outcrossing *E. fulgens* and *E. puniceoluteum* contain significantly more genetic diversity within than among populations. Indeed, low inbreeding levels and low genetic differentiation among populations were observed for both species (Pinheiro & al., 2010, 2011), in agreement with the general patterns observed for Mediterranean food-deceptive species (Cozzolino & Widmer, 2005; Scopece & al., 2010).

■ HYBRIDISATION AND BARRIERS TO GENE FLOW

Hybridisation is most likely an important mechanism of speciation in *Epidendrum*, as has been found for other organisms (Soltis & Soltis, 2009; Buggs & al., 2011). Gene flow between species showing different chromosome numbers was identified (Pinheiro & al., 2010, see below), and hybrids showing different chromosome numbers were found across different hybrid zones (Pinheiro & al., in prep.). Thus, different chromosome numbers do not seem to act as strong reproductive

barriers in *Epidendrum* as they do in other plant groups (Cozzolino & al., 2004; Marques & al., 2010).

In orchids, pre-mating barriers such as pollinator specificity have received significant attention from the scientific community, as many orchid groups are characterised by complex relationships with their pollinators (Van der Pijl & Dodson, 1966; Tremblay & al., 2005). Generally, *Epidendrum* is pollinated by a wide range of pollinator species and, apparently, lacks strong pre-mating barriers. Several Lepidoptera species were found visiting flowers of *E. fulgens* (Fuhro & al., 2010), *E. secundum* (Pansarin & Amaral, 2008), *E. densiflorum* (Pansarin, 2003) and *E. radicans* (Bierzychudek, 1981). Different species share an extensive number of pollinator species, such as *E. difforme* and *E. anceps* (Adams & Goss, 1976), *E. secundum* and *E. fulgens* (Pansarin & Amaral, 2008), and *E. anisatum* Lex. and *E. magnificum* Schltr. (Hágsater & Soto-Arenas, 2005). Given these observations, the high number of hybridisation events in the genus is not surprising (reviewed in Hágsater & Soto-Arenas, 2005). Individuals which are morphologically intermediate between putative parental species are commonly observed in sympatric populations (Fig. 4) (Dunsterville, 1979; Dressler, 1989; Hágsater & Soto-Arenas, 2005).

Despite morphological evidence for widespread hybridization in the genus, genetic confirmation of hybridization and an assessment of what types of hybrids are formed (F1, F2, backcrosses, etc.) is still limited. In this regard, the isolation and characterisation of specific molecular markers for *Epidendrum* may encourage research on topics such as hybridisation and speciation (Pinheiro & al., 2008a, b, 2009a, c). The use of highly informative nuclear and plastid microsatellites demonstrated a complex pattern of hybridisation between two *Epidendrum* species with different ploidy levels (*E. fulgens* $2n = 2x = 24$; *E. puniceoluteum* $2n = 4x = 52$). Hybridisation and late generation introgression were found in all six sympatric populations surveyed, suggesting that this process is a common phenomenon. Nuclear markers revealed the presence of F1 and late hybrid generations as well as signs of unidirectional introgression, demonstrating a high potential for interspecific gene flow even in the presence of different ploidy levels (Pinheiro & al., 2010). Indeed, unidirectional introgression was found to occur into the polyploid species, a pattern also observed in other plant groups (Chapman & Abbott, 2010), including orchids (Aagaard & al., 2005; Ståhlberg & Hedrén, 2009). According to Chapman & Abbott (2010), this unidirectional ‘transfer of adaptation’ from diploids to tetraploids by unidirectional introgression could increase the adaptability of tetraploids to diploid environments. The introgression between *E. fulgens* and *E. puniceoluteum* may have been facilitated by a high level of genome-wide synteny among different species due to their very recent radiation.

A broad ecological amplitude was detected in the hybrids, which were found growing in diverse habitats. This is in contrast with the strong genotype–habitat association found in the parental species, *E. fulgens* (only found in sand dune vegetation) and *E. puniceoluteum* (only found in seashore swamps). The different habitat associations detected among parental genotypes suggest that niche divergence contributes to species

cohesion as a potential post-mating, postzygotic barrier limiting gene flow (Pinheiro & al., 2010). Interspecific gene flow and introgression of fitness-related genes could enhance the ability to overcome habitat instability and adverse environmental conditions such as high levels of salinity, flooding, drought and lack of soil nutrients, which are habitat characteristics of *E. fulgens* and *E. puniceoluteum*. In addition, experimental crosses between parental species and between hybrids and *E. puniceoluteum* confirm unidirectional introgression into the polyploidy species (Pinheiro & al., 2010). The pattern of introgression between *E. fulgens* and *E. puniceoluteum* challenges the widely held view of ‘instant isolation’ among species of different ploidy level (Coyne & Orr, 2004) and opens interesting research possibilities investigating *Epidendrum* hybrid zones.

■ PHYLOGEOGRAPHY AND LINEAGE DIFFERENTIATION

Although next-generation-sequencing technologies open up exciting new possibilities in the field of phylogeography (Ekblom & Galindo, 2011), further conceptual developments will come from the strategic choice of studying non-model organisms and from the synthesis of comparative information across different regions of the globe. In this context, many empirical

phylogeographic surveys in tropical countries are needed because most of the current studies are concentrated in temperate regions (Beheregaray, 2008). The extensive morphological diversification and different chromosome numbers found among different populations make *Epidendrum* species excellent models to test the influence of historical demographic events on lineage diversification and speciation in the Neotropical region.

Several regional or landscape genetic studies have compared phylogeographic results across multiple co-distributed species (Bermingham & Moritz, 1998). This approach is especially recommended for complex scenarios of diversification, such as those found in the Neotropics (Antonelli & Sanmartín, 2011). The idea behind comparative phylogeographic studies is that species with similar ranges or ecologies often tend to be genetically structured in similar ways. Many *Epidendrum* species have a broad geographic distribution and ecological preferences, covering different biomes and habitats. Moreover, epiphytes, rupicolous and terrestrial co-occurring species could give insight into evolutionary processes at the community level (Avice, 2009). In this regard, *E. secundum*, *E. densiflorum*, *E. latilabre* Lindl., *E. dendrobioides* Thunb., *E. flexuosum* and *E. radicans* represent suitable species for developing this type of comparative studies. Furthermore, in *Epidendrum*, many species complexes vary widely in their ecology encompassing rain forest, grassland and sand dune species thus offering the

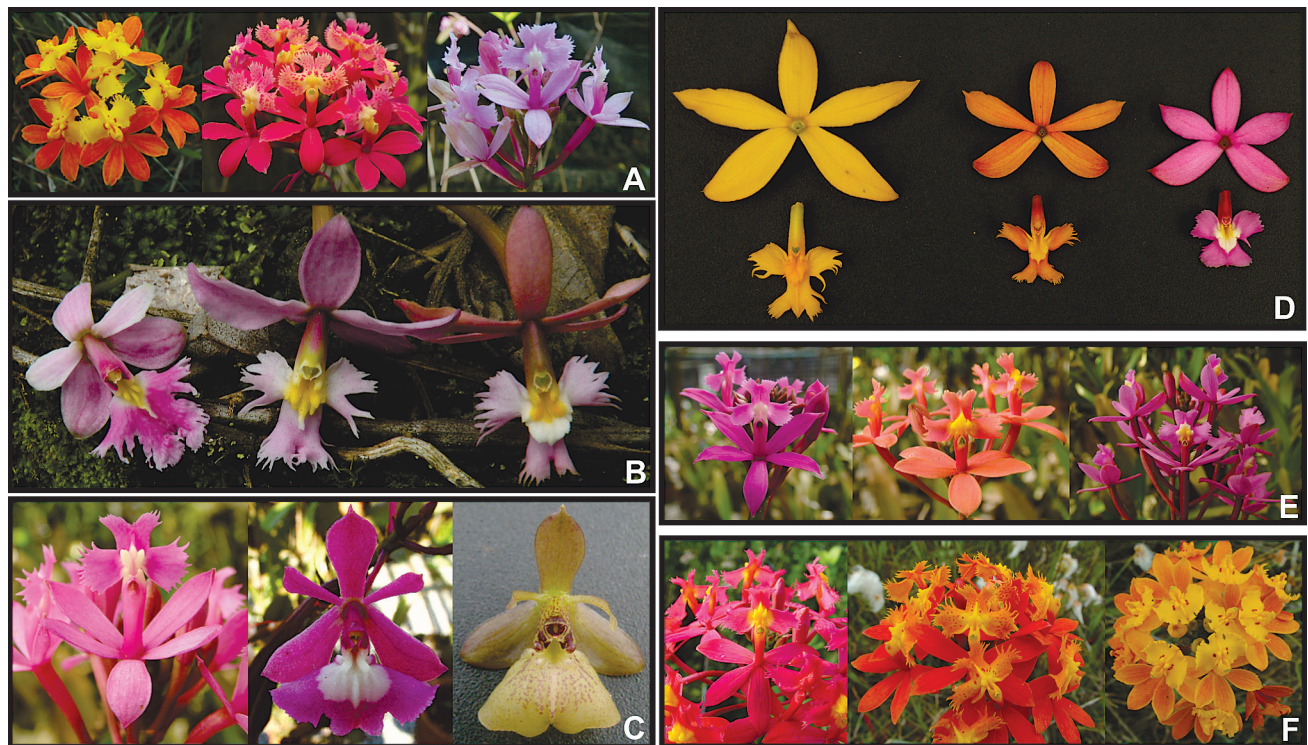


Fig. 4. Putative hybrids between different *Epidendrum* species pairs of subgenus *Amphyglottium*, found in natural sympatric populations. In each block, the central flower represents the putative hybrid. **A**, Hybrid between *E. fulgens* Brongn. (left) and *E. denticulatum* Barb. Rodr. (right); **B**, hybrid between *E. calanthum* Rchb. f. & Warsz. (left) and *E. secundum* Jacq. (right); **C**, *E. purpureum* Barb. Rodr. (center) is a putative hybrid species between *E. denticulatum* (left) and *E. myrmecophorum* Barb. Rodr. (right); **D**, hybrid between *E. xanthinum* Lindl. (left) and *E. secundum* (right); **E**, hybrid between *E. denticulatum* (left) and *E. secundum* (right); **F**, hybrid between *E. puniceoluteum* Pinheiro & Barros (left) and *E. fulgens* (right).

opportunity for comparative phylogeographic studies among related species with very similar life-cycles but different distributional ranges.

To date, several phylogeographical studies in the Neotropical region have focused on organisms from core rain forest habitats (Palma-Silva & al., 2009; Ribeiro & al., 2011). Given this unequal distribution of studies across forest and grassland organisms, it is not surprising that refugia have primarily been identified for species associated with forest environments. However, contrary to the pattern observed for forest species, grassland and sand dune plants expanded their geographic limits during glacial cycles, and contracted their range during warm periods, when interglacial refuges were formed (Bennett & Provan, 2008).

Phylogeographic results for *E. fulgens* (Pinheiro & al., 2011) confirmed the patterns expected for grassland and sand dune plants. This species is distributed in seashore vegetation in southeastern Brazil, growing in sand dune vegetation and on granitic rock outcrops. Phylogeographic breaks were consistent with the limits between different biomes (Brazilian Atlantic Rainforest and Pampas) and show substantial agreement with earlier floristic inventories (Rambo, 1950). Recent reductions of population size (bottlenecks) were detected in northern populations. This type of demographic change was not expected for those populations, as earlier studies of forest species had found evidence of population expansion in the same areas (Palma-Silva & al., 2009; Ribeiro & al., 2011). While northern populations showed reduced levels of genetic

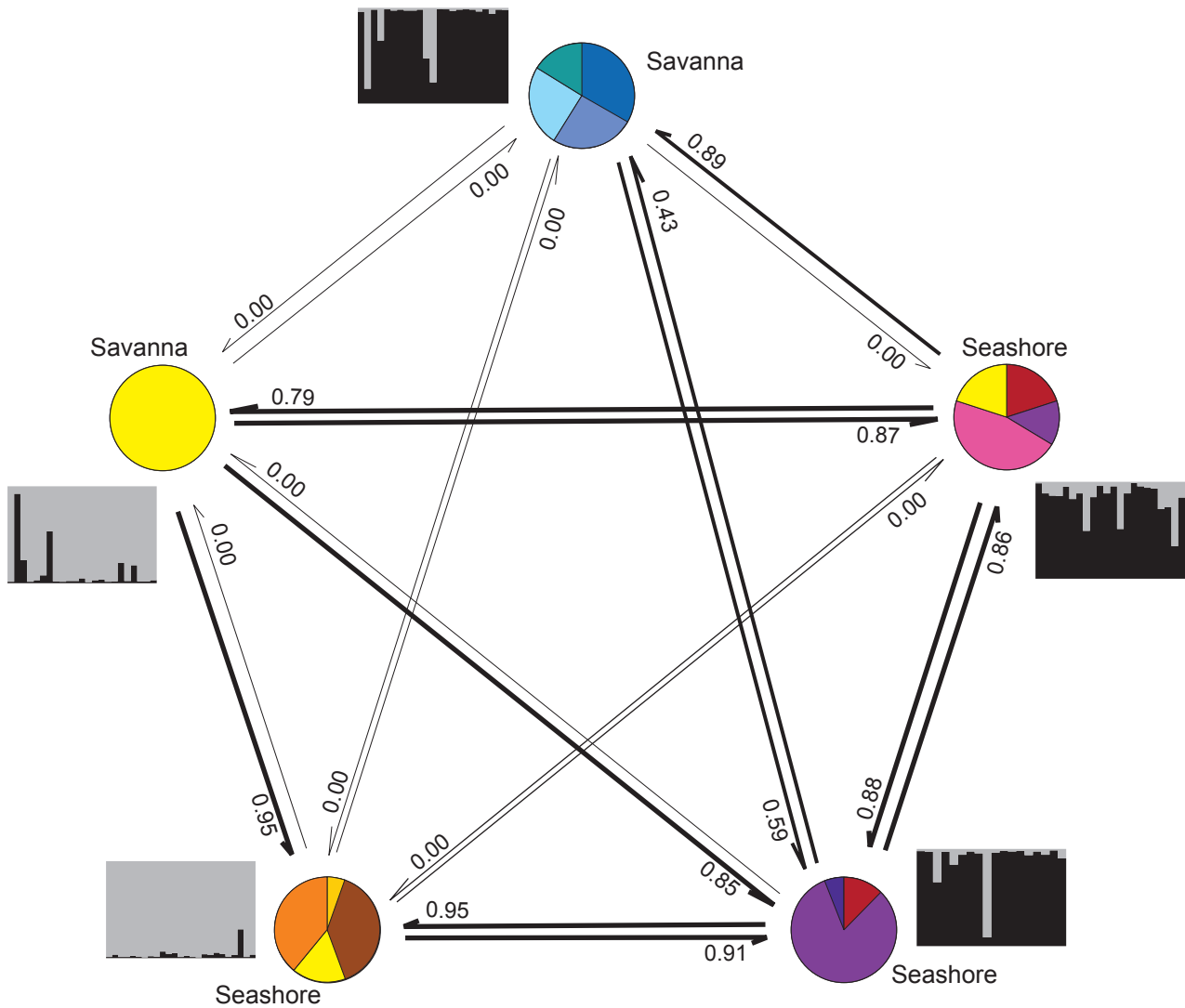


Fig. 5. Reproductive isolation between *E. denticulatum* populations (from Pinheiro & al., in prep.) occurring in distinct types of vegetation (savanna and seashore sand dune vegetations). Arrows point to the maternal parents in the crossing experiments. Arrow width is proportional to cross-compatibility, and numbers indicate the percentage of seed viability found following experimental crosses. Pie charts indicate haplotype frequencies in each population of *E. denticulatum*. Bar charts show assignment results obtained by STRUCTURE, where each vertical bar represents an individual, and the proportion of colour in each bar represents an individual's assignment probability to the different groups found (grey and black).

diversity, southern populations displayed higher levels of both plastid and nuclear diversity, in accordance with patterns observed for other sand dune and grassland species (Jakob & al., 2009; Cosacov & al., 2010). The increasing number of studies using grassland and sand dune species is revealing unexpected phylogeographic patterns such as genetic signatures of cryptic refuges, past altitudinal movements and interglacial population stasis (Bennett & Provan, 2008; Provan & Bennett, 2008; Stewart & al., 2010), indicating the importance of these organisms for future studies in the Neotropical region.

Past climatic fluctuations also have impacted genetic diversity and the reproductive compatibility among populations of *E. denticulatum* (Pinheiro & al., in prep.). This species is distributed in southeastern and northeastern Brazil, occurring across different biomes, such as the Tabuleiro Forest, the Seasonal Semideciduous Forest and seashore sand dunes. As expected, populations from different biomes showed deep phylogeographic splits and contrasting levels of genetic diversity. In this context, different populations may contain specific combinations of alleles such that there is high fertility within populations and genetic groups but partial and asymmetric sterility in most interpopulation crosses (Fig. 5). The diversity of seed viability found in *E. denticulatum* clearly suggests that multiple genetic mechanisms are in operation, at both pre- and/or postzygotic stages. A substantial portion of reproductive isolation is also the results of selection for divergent habitats (Pinheiro & al., 2010), and reciprocal transplant experiments could provide important estimates of immigrant and resident viability for each population to determine the divergent adaptation that results in immigrant inviability (Nosil & al., 2005). Furthermore, new sequencing technologies open a promising venue to study the dynamics of porous genomes in non-model organisms, enabling researchers to identify the particular genomic regions and genes responsible for reproductive isolation barriers in plants (Lexer & Widmer, 2008; Twyford & Ennos, 2012). The permeability of reproductive barriers and its variability within species, in a phylogeographic context, could represent an interesting avenue for future research in *Epidendrum*.

■ CONCLUSIONS AND FUTURE REMARKS

Much of our knowledge about evolution and the underlying ecological and molecular processes has been derived from the studies of model, or reference, organisms (Müller & Grossniklaus, 2010). However, species-rich groups such as orchids remain under-represented in studies of speciation, evolution and reproductive isolation as well as in the development of molecular markers relative to other economically important plant families (Peakall, 2007).

The increasing amount of detailed knowledge about *Epidendrum* provides a rich source of interesting but unresolved questions in plant ecology and plant evolution in the Neotropical region. The recent isolation and characterisation of specific molecular markers for *Epidendrum* will speed up obtaining knowledge of the group, as already noted by Peakall (2007). Time is ripe to ...

... perform genome-wide investigations into patterns of nucleotide diversity within and among *Epidendrum* species. In such way taxonomic uncertainties and the phylogenetic position of most species should be clarified, and barcoding projects should consider *Epidendrum* as a target group.

... describe the population structure and evolutionary history of species for evaluating the influence of selective forces and demography on ecologically important candidate genes associated with the resistance of adverse abiotic conditions such as drought, salinity and flooding.

... correlate the evolution of pollination strategies with the permeability of reproductive barriers and the amount and effect of inter- and intraspecific gene flow.

... identify the adaptive ecological significance of trait variation and hybridisation. Long-term field experiments, including reciprocal transplants and reproductive manipulations will provide direct tests of adaptive processes and evidence for habitat selection.

... test the long-standing hypothesis of species differentiation through founder effects in the Neotropical region (Gentry & Dodson, 1987).

With the increasing availability of genetic tools, allowing for the generation of huge amounts of molecular data in a very cost-effective way, further progress in *Epidendrum* research will take place across the range of fundamental questions outlined here. By embracing a combination of approaches, the investigation of *Epidendrum* species will continue to provide unique opportunities to expand and integrate our understanding of ecology, evolution and genomics in the origin of Neotropical biodiversity.

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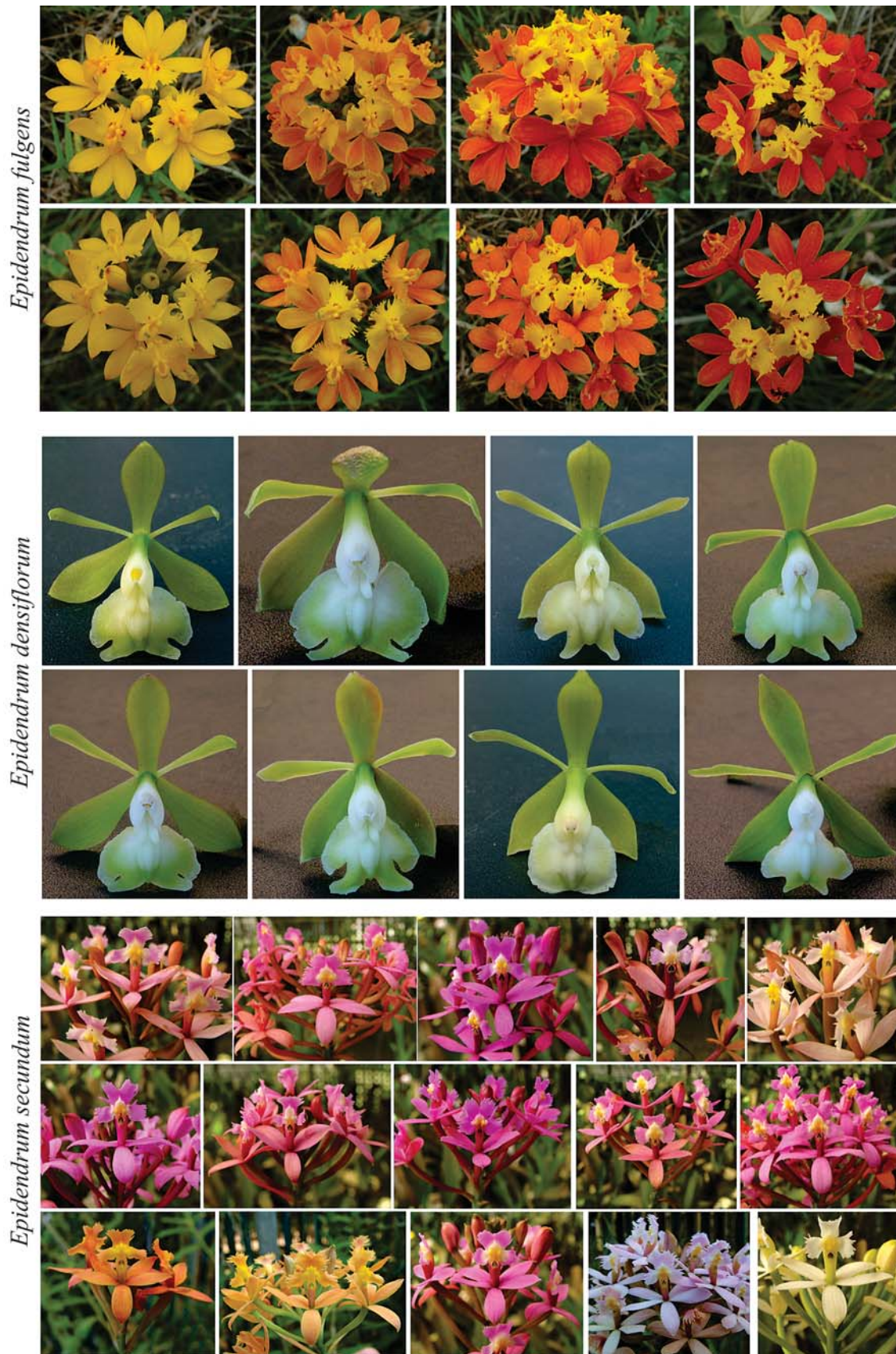


Fig. S1. Morphological variation of flowers in individuals of *Epidendrum fulgens* (Ilha do Cardoso – SP, Brazil), *E. densiflorum* Hook. (Ilha do Cardoso – SP, Brazil) and *E. secundum* Jacq. (Nova Friburgo – RJ, Brazil).