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# Evolutionary diversification and historical biogeography of orchidaceae in Central America with emphasis on Costa Rica and Panama

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# Evolutionary diversification and biogeography of *Lepanthes* and allies

# Chapter 8

## Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot

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**Abstract.** The Andean mountains of South America are the most species-rich biodiversity hotspot worldwide with c. 15% of the world's plant species, in only 1% of the world's land surface. Orchids are a key element of the Andean flora, and one of the most prominent components of the Neotropical epiphyte diversity, yet very little is known about their origin and diversification. We address this knowledge gap by inferring the biogeographical history and diversification dynamics of the two largest Neotropical orchid groups (Cymbidieae and Pleurothallidinae), using two unparalleled, densely sampled orchid phylogenies (including more than 400 newly generated DNA sequences), comparative phylogenetic methods, geological and biological datasets. We find that the majority of Andean orchid lineages only originated in the last 20–15 Ma. Andean lineages are derived from lowland Amazonian ancestors, with additional contributions from Central America and the Antilles. Species diversification is correlated with Andean orogeny, and multiple migrations and recolonizations across the Andes indicate that mountains do not constrain orchid dispersal over long timescales. Our study sheds new light on the timing and geography of a major Neotropical diversification, and suggests that mountain uplift promotes species diversification across all elevational zones.

# 8.1 Introduction

Species richness is unevenly distributed in time (Simpson, 1953), space (Willis, 1922) and across the Tree of Life (Vargas and Zardoya, 2014). An understanding of the processes underlying current patterns in species richness and distribution therefore constitutes a major scientific challenge. The Andean mountains of South America contain c. 15% of the world's plant species, in only 1% of the world's land surface, resulting in the most species-rich biodiversity hotspot worldwide (Myers et al., 2000). A large proportion of this diversity is found in high-altitude grasslands, and is suggested to have resulted from recent rapid speciation events (Hughes and Eastwood, 2006; Hughes and Atchison, 2015). By contrast, Andean seasonally dry forests experienced much slower diversification and have older origins (Pennington et al., 2010), suggesting contrasted macroevolutionary histories within the Andean biodiversity hotspot (Pennington et al., 2010; ter Steege et al., 2013; Valencia et al., 1994).

In a seminal paper, Gentry (1982) postulated that mountain uplift was a major trigger of Andean mega-diversity, although he posited that this might have occurred indirectly via biotic interactions. A pivotal result of Gentry's floristic analyses was the discovery of two patterns of plant distribution in the Neotropics: 'Amazonian-centred' and 'Andean-centred' taxa. Amazonian-centred taxa consist mostly of canopy trees and lianas, whereas Andean-centred taxa are almost exclusively epiphytes and shrubs (Gentry, 1982). The latter occur mostly in the Northern Andes, with secondary centres in the Brazilian coastal mountains and Central America, together accounting for c. 33% of all Neotropical plants (Gentry, 1982), and thus largely contributing to the world's most species-rich biodiversity hotspot, the tropical Andes (Myers *et al.*, 2000).

Contrasting with the dominant views at the time, Gentry (1982) hypothesized that the Andean-centred flora resulted from 'recent, very dynamic speciation', a hypothesis that we test here. Gentry and Dodson (1987) further suggested that the high diversity of epiphytes in the Northern Andes and southern South America could have resulted from the finer niche partitioning in these forests, allowing for high alpha diversity, the high microsite differentiation of mountain areas, fostering high beta diversity, and explosive speciation driven by genetic founder effects because of the environmental dynamicity, implying frequent relocation. Orchids are one of the most characteristic and diverse components of the Andean flora (Gentry and Dodson, 1987; Krömer and Gradstein, 2003; Parra-Sánchez et al., 2016; Richter et al., 2009). They often make up 30–50% of the total epiphytic species number reported along the Northern Andes (Kreft et al., 2004; Küper et al., 2004), and epiphytic orchids account for 69% of all vascular epiphytes world-wide (Zotz and Winkler, 2013). Neotropical epiphytic orchids are generally characterized by narrowly restricted populations with small numbers of individuals (Crain and Tremblay, 2012; Jost, 2004; Pandey et al., 2013; Tremblay and Ackerman, 2001). Despite the ecological importance and prominence of epiphytic orchids (and of epiphyte diversity overall) in the Andean flora, their origin and diversification have not been explicitly studied because of the difficulties in generating densely sampled and strongly supported phylogenies. We address these issues by studying the evolutionary history of the two largest Neotropical orchid clades, namely Cymbidieae and Pleurothallidinae. The Cymbidieae comprise over 3700 species, 90% of which occur in the Neotropics (the remaining species occur in tropical Africa and Australasia). Cymbidieae comprise 12 subtribes, four of which are the most

speciose and include Andean-dwelling subclades (i.e. Maxillariinae, Oncidiinae, Stanhopeinae and Zygopetalinae; Pridgeon *et al*., 2009). Pleurothallidinae comprise 44 genera and 5100 exclusively Neotropical species (Karremans, 2016) distributed mostly in the highlands of the Northern Andes and Central America. Together, they are the most representative elements of the Andean orchid flora (Pérez-Escobar *et al.,* 2009; Pridgeon *et al.*, 2009; Kolanowska, 2014) and make up most of their species richness. In addition, these lineages have evolved a rich array of pollination syndromes and mating systems (including protandry, unisexuality, cleistogamy; Gerlach and Schill, 1991; Borba *et al.*, 2011; Pérez-Escobar *et al.*, 2016a) that have long fascinated botanists and naturalists (Darwin, 1877; Lindley, 1843). This is particularly true for Cymbidieae, in which up to seven pollination syndromes have been recorded (Pridgeon et al., 2009; van der Cingel, 2001), ranging from species exclusively pollinated by male euglossine bees (Ramírez et al., 2011) to those pollinated only by oil bees. Data on the pollination ecology of Pleurothallidinae are very scarce, but scattered reports across the clade suggest that they are mostly pollinated by a vast array of dipteran lineages (Blanco and Barboza, 2005; Pupulin et al., 2012). Rapid Andean orogeny could have promoted orchid species richness by creating ecological opportunities, such as increasing the landscape, mediating local climate change, creating novel habitats and forming insular environments that affected migrations and allopatric speciation through isolation (Gentry and Dodson, 1987; Hoorn et al., 2013). This effect should have been most accentuated over the last 10 Ma, during which c. 60% of the current elevation of the Andes was achieved (Gregory-Wodzicki, 2000). Diversification studies of Andean centred clades have provided evidence for rapid diversification that temporally matches the Andean surface uplift, for instance in the plant genera *Lupinus*, *Espeletia*, *Halenia* and *Heliotropium*, and in the families Campanulaceae and Annonaceae (von Hagen and Kadereit, 2003; Bell and Donoghue, 2005; Donoghue and Winkworth, 2005; Hughes and Eastwood, 2006; Pirie *et al.*, 2006; Antonelli *et al.*, 2009b; Luebert *et al.*, 2011; Drummond *et al.*, 2012; Madriñán *et al.*, 2013; Lagomarsino *et al.*, 2016; Diazgranados and Barber, 2017). Taken together, these studies suggest that rapid Andean uplift yielded new niches that fostered both adaptive and non-adaptive radiations (Nevado et al., 2016). Other Andean groups, such as hummingbirds, diversified mostly before Andean uplift (McGuire et al., 2014) or after it had attained most of its current height (Smith et al., 2014). We address the impact of the Andean uplift on the diversity and distribution of orchids by inferring the dynamics of speciation, extinction and migration, whilst simultaneously incorporating surface uplift of the two largest Andean Neotropical orchid clades Cymbidieae and Pleurothallidinae. We rely on model-based inference methods in historical biogeography, ancestral area and character estimation approaches, and a series of diversification analyses to investigate the following questions. From which geographical area(s) do Andean orchids mostly originate? Is there evidence for the Andes acting as a dispersal barrier for epiphytic lowland taxa? Did the Andean uplift enhance orchid diversification and, if so, was this effect evident on all species from the Andean region or just those from the highest elevations? Is Andean diversity derived from pre-adapted (i.e. high elevation) lineages or rather descendants of lowland migrants (either local or from other areas)? In addition, we use the limited available data to evaluate whether shifts in pollination syndromes are associated with changes in diversification rates. Our results support Gentry's prediction (Gentry, 1982) that Andean-centred groups have resulted from recent rapid speciation, suggesting that Andean

orogeny provided opportunities for rapid orchid species diversification in the world's premier plant biodiversity hotspot. Such diversity is derived from lowland lineages but, more rarely, from migrants already pre-adapted to cool environments, a more frequent situation documented from other mountain environments (Merckx et al., 2015).

# 8.2 Materials and Methods

## 8.2.1 Taxon sampling, DNA sequencing and phylogenetic analysis

To generate solid phylogenies of the tribe Cymbidieae and subtribe Pleurothallidinae, we newly generated a total of 420 sequences of the nuclear ribosomal internal transcribed spacer (ITS) and a c. 1500-bp fragment of the gene ycf 1 of underrepresented lineages of key biogeographical importance. DNA amplification, PCR product purification and sequencing were conducted as described previously in Irimia *et al.* (2014) and Pérez-Escobar *et al.* (2016a). Voucher information and GenBank accession numbers are provided in Supporting Information Tables 8.S1 and 8.S2. We merged our novel dataset with previously generated data from the studies of Blanco *et al.* (2007), Whitten *et al.* (2014), Karremans *et al.*, (2016a,b), and Ramírez *et al.* (2011), using the R-package Megaptera v.1.0 (available at https://github.com/cran/megaptera.git). We retrieved 3541 sequences of nuclear (ITS) and plastid (matK, trnL-F region, psbA, ycf1). We selected outgroup taxa representing the old and new world subtribes Polystachyinae, Aeridinae and Laeliinae. Trees were rooted on *Calypso bulbosa* (for Cymbidieae) and *Arpophyllum giganteum* (for Pleurothallidinae) following Whitten *et al.* (2014). Poorly aligned positions were excluded from the alignments using GBLOCKS v.0.9 (Talavera and Castresana, 2007). To statistically detect potential incongruences between plastid and nuclear DNA phylogenies, we used the tool Procrustes Aapproach to Cophylogeny (PACo; http://www.uv.es/cophylpaco/) (Balbuena *et al.*, 2013; Pérez-Escobar *et al.*, 2016b). Maximum likelihood (ML) tree inference was performed using RAXML-HPC v.8.0 (Stamatakis, 2014), under the GTR + G substitution model with four gamma categories (best model for both datasets as inferred via the Akaike information criterion (AIC) in jModelTest v.2.1.6; Darriba *et al.*, 2012), with 1000 bootstrap replicates and data partitioning by genome compartment. All phylogenetic and dating analyses were performed in the CIPRES Science Gateway computing facility (Miller et al., 2015).

## 8.2.2 Molecular clock dating

A few unambiguous orchid macrofossils are available for Orchidaceae (*Dendrobium winikaphyllum*, *Earina fouldenensis*, *Meliorchis caribea*; Ramírez *et al.*, 2007; Conran *et al.*, 2009), but these are assigned to lineages very distantly related to our groups of interest. Using distant outgroups to calibrate our Cymbidieae and Pleurothallidinae phylogenies would have created extensive sampling heterogeneities, which can result in spurious results (Drummond and Bouckaert, 2014). Thus, we had to rely on secondary calibrations. In order to obtain the best secondary calibration points possible, we first generated an Orchidaceae-wide, fossil-calibrated phylogeny, sampling 316 orchid species and four loci (nrITS, matK, rbcL and trnL-F), sampled as evenly as possible along the tree. Detailed settings and fossil calibrations used to generate an Orchidaceae-wide phylogeny are provided in the extended Methods 8.S1.

Secondary calibration points were obtained from our Orchidaceae-wide dated phylogeny, and the most recent common ancestor (MRCA) of Cymbidieae + Vandeae was dated to 34±7 Ma, 95% credible interval (CI), whereas that of Pleurothallidinae + Laeliinae was estimated to 20±7 Ma. We therefore used a normal prior (with values of mean = 34, SD = 4 for Cymbidieae; mean = 20, SD = 3 for Pleurothallidinae, to reflect the 95% CI from our fossil-calibrated tree) to calibrate our phylogenies using these secondary constraints, which were designed to reflect the uncertainty previously estimated for the root node of Cymbidieae and Pleurothallidinae.

## 8.2.3 Ancestral range estimation

Species ranges were coded from the literature (Pridgeon *et al.*, 2009) and from herbarium specimens through a survey of virtual collections and loans of several herbaria (AMES, COL, F, MO, SEL, US, M), as well as the Global Biodiversity Information Facility (GBIF) repository. To query the GBIF database, we relied on the function "occ" of the R-package SPOCC (Chamberlain et al., 2016). A total of 19,486 distribution records were compiled for the Cymbidieae, and 9042 records for the Pleurothallidinae. Protocols for distribution maps and species richness pattern analyses are detailed in Methods S1. Distribution maps for Cymbidieae and Pleurothallidinae (summarized in Figs. 8.S1, 8.S2) and extant distribution patterns identified for other plant lineages (e.g. Rubiaceae, Antonelli *et al.*, 2009b) allowed the identification of 10 main distribution areas (see the inset in Figs. 8.1, 8.2). Species were assigned to one of these regions: Central America (comprising southern Florida to Panama); West Indies (i.e. Caribbean Islands); Northern Andes (mountain ranges from elevations higher than 500 m in Colombia and Venezuela); Central Andes (from Peru to the Tropic of Capricorn, from elevations higher than 500 m); Amazonia (including lowlands and montane forest below 500 m in Colombia, Ecuador, Peru, Brazil, Venezuela, Guyana, Suriname and French Guiana); the Guiana Shield (including elevations higher than 500 m in north-eastern South America (Brazil, Guyana, Suriname and Venezuela)); South-eastern South America (including the Brazilian shield, but also lowlands in eastern Brazil and northern Argentina); Chocó (comprises lowlands below 500 m of the western Andes in Colombia and Ecuador); Africa; and Australasia. To infer the ancestral range of all examined lineages in Cymbidieae and Pleurothallidinae, we used the R-package BioGeoBEARS v.0.2.1 (Matzke, 2014, 2013). In addition, in order to estimate the number of migrations, dispersals, extinctions and within-area speciation events from our phylogeny, we used biogeographical stochastic mapping (BSM) (Matzke, 2014) under the best-fit model, as implemented in BioGeoBEARS (for detailed settings, see Methods 8.S1).

## 8.2.4 Rates of species diversification

To infer the diversification dynamics of the Cymbidieae and Pleurothallidinae, we first used a time-dependent model implemented in BAMM v.2.5.0 (Rabosky, 2014) to estimate the rates of extinction and speciation across the phylogenies. Incomplete taxon sampling was accounted for by assigning a sampling fraction of 25% of the extant orchid diversity of Cymbidieae, and 13% of Pleurothallidinae (sampling fractions of every genus sampled were incorporated according to Chase *et al.*, 2015). We performed three runs with 1 million Markov chain Monte Carlo (MCMC) generations, sampling parameters every 10,000 generations. Diversification rates and

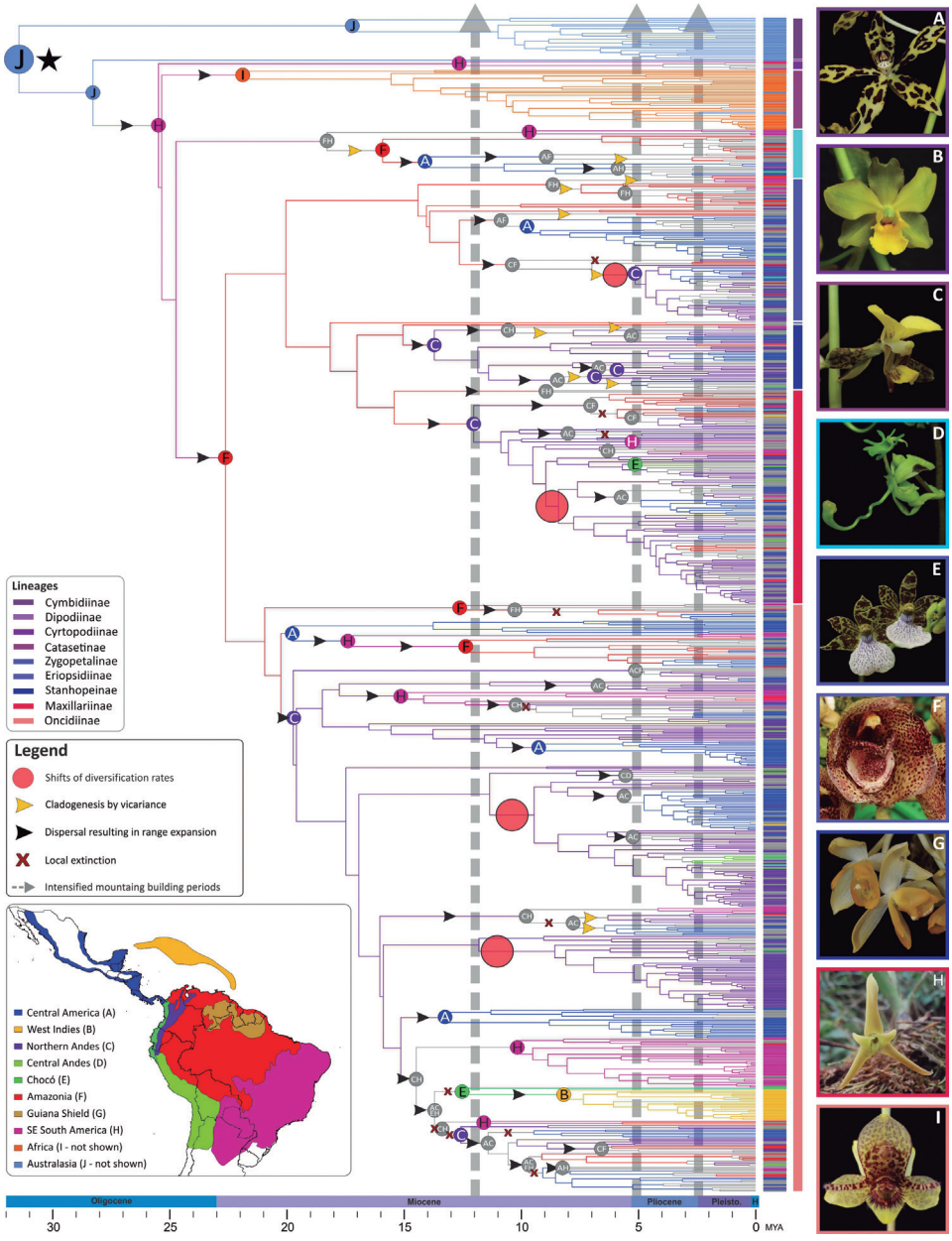


**Figure 8.1.** Biogeographical history of Cymbidieae orchids. Letters on the coloured circles at the nodes indicate the estimated ancestral area with the highest probability as inferred by BIOGEOBEARS. Branches are colour coded following the reconstructed area of their corresponding node, and the geographical ranges of every taxon are shown as vertical bars in front of the terminals. The black star indicates the most recent common ancestor of Cymbidieae. Grey arrows show the periods of accelerated Andean uplift (Gregory-Wodzicki, 2000).Changes on shifts of diversification rates are shown as pale red circles on the

rate shift configurations were plotted using the R-package BAMMtools (Rabosky *et al*., 2014). We checked the convergence of the runs by plotting the loglikelihood across MCMC generations sampled in the 'mcmc_out' file. To evaluate the best model generated by BAMM (compared with a null $M_0$ model with no diversification rate shifts), we relied on Bayes Factors calculated with the *computebayesfactor* function of BAMMtools. We examined the 95% credible set of macro-evolutionary shift configurations using the BAMMtools function *credibleShiftSet*. We sought cross validation of our BAMM results with RPANDA (Morlon et al., 2016), and details about the settings are provided in Methods S1.

## 8.2.5 Geographical state-dependent analyses

We used GeoSSE (Goldberg et al., 2011), an extension of the BiSSE model that allows lineages to occur simultaneously in two areas and to test whether one area has overall higher speciation rates, as implemented in the R-package Diversitree v.0.9-7 (FitzJohn, 2012). To test whether lineages restricted to the Northern Andes ('A') had higher diversification rates than lineages absent from the Northern Andes (collectively called 'B' here), we used Bayesian MCMC GeoSSE analyses of 1 million generations on the maximum clade credibility tree from BEAST (in the particular case of Cymbidieae, only Neotropical representatives were included). Implemented models in GeoSSE and settings of tailored simulations to account for Type I error biases in GeoSSE are provided in Methods S1.

## 8.2.6 Mapping speciation rates in the Neotropics

Based on the speciation and extinction rates inferred for orchid lineages, and their geographical occurrence, it is possible to identify important areas of diversification as plotted on a heat map (Condamine et al., 2013). For this purpose, we designed a novel method that involves retrieving speciation rates from BAMM analyses using the function *GetTipsRates* in BAMMtools v.2.1 (Rabosky et al., 2014a) and to link them to species occurrences. Rates were further associated to known distribution records of Cymbidieae and Pleurothallidinae and interpolated to a polygon representing the currently known distribution of Cymbidieae and Pleurothallidinae species, using the inverse distance weight method implemented in the software ARCMap v.9.3 (Esri). To account for geographical sampling biases, we divided the geographical range of species records into a grid of 0.5° 9 0.5° cells. We then randomly sampled occurrences arrayed on every grid cell using the R package Raster (Hijmans and Elith, 2016), so that a single occurrence per grid cell was kept.

branches. Range expansions, local extinctions and cladogenetic events via vicariance are indicated on the branches with black and yellow arrow heads and red crosses, respectively. Subtribe members of Cymbidieae are colour coded. Right panels showselected representatives of **A.** Cymbidiinae (*Grammatophyllum measuresianum*); **B.** Cyrtopodiinae (*Cyrtopodium macrobulbon*; photograph by D. Bogarín); **C.** Eulophiinae (*Eulophia streptopetala*); **D.** Catasetinae (*Cycnoches egertonianum*); **E.** Zygopetalinae (*Zygopetalum* aff. *brachypetalum*); **F.** Coeliopsidinae (*Peristeria cerina*); **G.** Stanhopeinae (*Sievenkingia* sp.); **H.** Maxillariinae (*Cryptocentrum sp*.); **I.** Oncidiinae (*Trichoceros* sp.). Photographs (except B): O. Pérez. (Inset) Coded areas for biogeographical analysis. Political divisions obtained from DIVA-GIS (http://www.diva-gis.org/gdata). Timescale shown at bottom is expressed in million years ago(Ma).

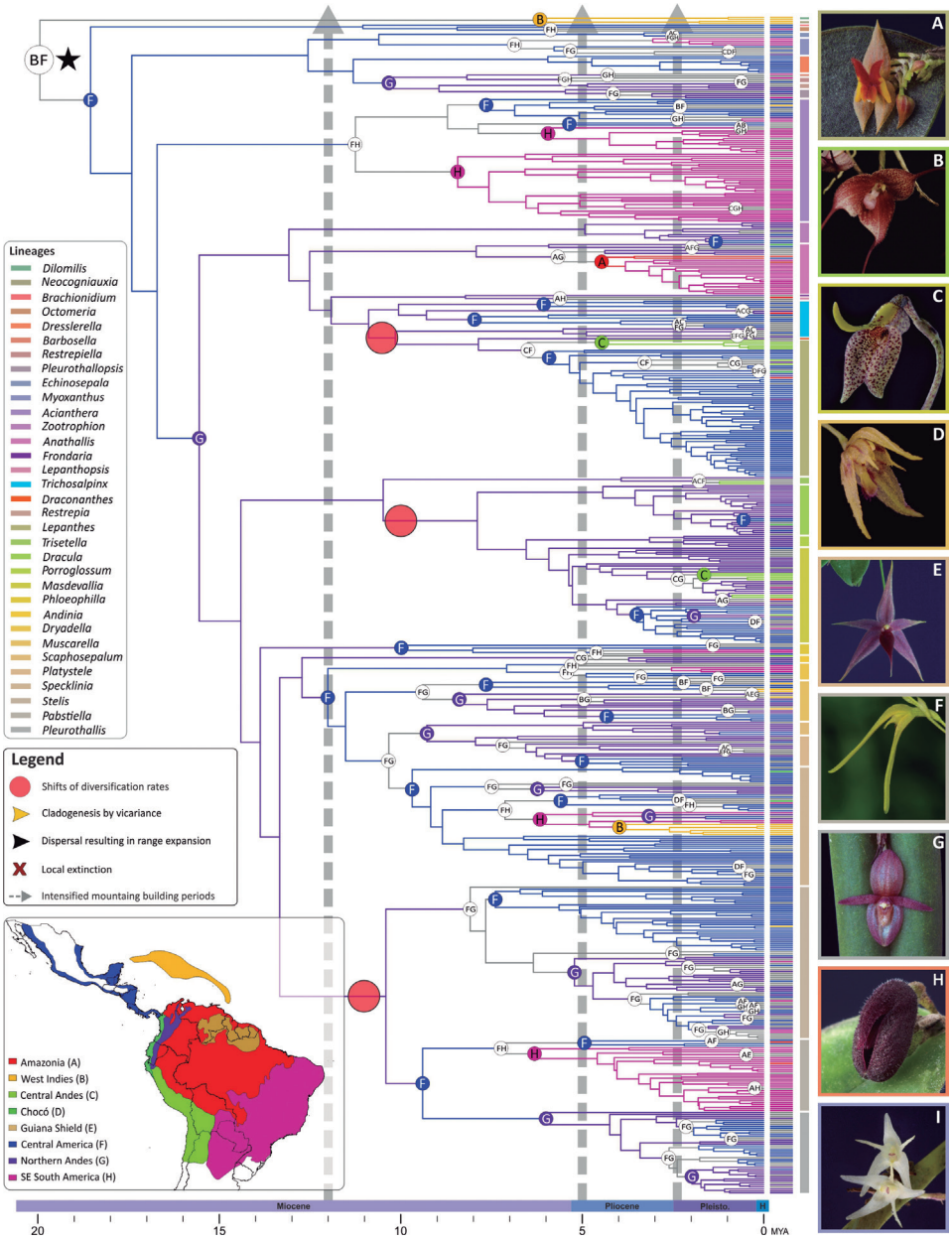


**Figure 8.2.** Biogeographical history of Pleurothallidinae orchids. Letters on coloured circles at the nodes indicate the estimated ancestral area with the highest probability as inferred by BIOGEOBEARS. Branches are colour coded following the reconstructed area of their corresponding node, and geographical ranges of every taxon are shown as vertical bars in front of the terminals. The black star indicates the most recent common ancestor of Pleurothallidinae. Grey arrows show the periods of accelerated Andean uplift (Gregory-Wodzicki, 2000). Changes on shifts of diversification rates are shown as pale red circles on the

## 8.2.7 Palaeo-elevation-dependent diversification

We tested the effect of past environmental change on the diversification of Cymbidieae and Pleurothallidinae using birth–death models that allow speciation and extinction rates to vary according to a quantitative, time-dependent, environmental variable (Condamine *et al.*, 2013), here the palaeo-elevation of the Northern Andes (Hoorn *et al.*, 2010; Lagomarsino *et al.*, 2016). The R-package PSPLINE (Ramsey and Ripley, 2010) was used to interpolate a smooth line for Andean palaeo-elevation. This smooth line was sampled during each birth–death modelling process to give the value of the palaeo-elevation variable at each time point. Speciation and extinction rates were then estimated as a function of these values along the time-calibrated phylogenies, according to the parameters of each model. The palaeo environmental dependent model is implemented in the Rpackage RPANDA v.1.1 (Morlon et al., 2016). Implemented models in RPANDA are provided in Methods S1.

## 8.2.8 Ancestral character state estimation

To account for potential biotic variables as drivers of Neotropical orchid diversification, such as shifts on pollination syndromes (Givnish et al., 2015), we compiled information on the pollination syndromes of Cymbidieae from the literature (Gerlach, 2011; Pansarin et al., 2009; Pridgeon et al., 2009; Ramirez et al., 2011; Singer, 2002; van der Cingel, 2001), and consulted experts on specific groups (see the Acknowledgements section). As a result of a dearth of detailed information on pollination ecology (i.e. available for c. 6% of taxa sampled only), we followed a generalist coding approach, and seven pollination syndromes, (i.e. bee, bird, butterfly, lepidopteran, fly, wasp and self-pollination) were coded. To account for missing information on pollination syndromes, we assigned equal probabilities to all character states to taxa with unknown pollination syndromes. To estimate ancestral elevation ranges in Pleurothallidinae and Cymbidieae, we obtained absolute elevation values from herbarium records for every taxon sampled in our phylogenies. We obtained a mean of five values per taxa sampled, and we coded mean elevation values as a continuous character. We followed the classification of major Andean ecoregions proposed by Rangel-Churio *et al.* (1997) and Jørgensen and León-Yánez (1999), and taxa occurring at elevations higher than 1100 m were considered to inhabit sub-Andean (montane) forests (1100–2400 m). Species occurring at elevations of < 1100 m were considered as lowland inhabitants. Detailed settings for ancestral character state of altitude and pollination syndromes are provided in Methods 8.S1.

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branches. Range expansions, local extinctions and cladogenetic events via vicariance are indicated on the branches with black and yellow arrowheads and red crosses, respectively. Generic members of Pleurothallidinae are colour coded. Right panels show selected representatives of **A.** *Lepanthes* (*Lepanthes* sp.); **B.** *Dracula* (*D. astuta*); **C.** *Masdevallia* (*M. utriculata*); **D.** *Muscarella* (*M. exesilabia*); **E.** *Platystele* (*P. porquinqua*); **F.** *Pabstiella* (*P. ephemera*); **G.** *Pleurothallis* (*P. adventurae*); **H.** *Dresslerella* (*D. pilosissima*); **I.** *Myoxanthus* (*M. colothrix*). Photographs: A. Karremans, D. Bogarín and O. Pérez. (Inset) Coded areas for biogeographical analysis. Political divisions obtained from DIVA-GIS (http://www.diva-gis.org/gdata). Timescale shown at bottom is expressed in million years ago, Ma.

# 8.3 Results

## 8.3.1 Phylogenetics, age and biogeography of Andean orchids

Analyses of phylogenetic incongruence detection identified 259 and 125 potential conflicting tips in Cymbidieae and Pleurothallidinae, respectively (Figs. 8.S3, 8.S4), all of which clustered in weakly to moderately supported clades (< 75% bootstrap support, BS) or in clades with extremely long branches. These analyses indicated the absence of supported phylogenetic incongruence (Mason-Gamer and Kellog, 1996; Pérez-Escobar *et al.*, 2016b). In the absence of supported phylogenetic conflicts, nuclear and plastid partitions of Cymbidieae and Pleurothallidinae were concatenated. For the Cymbidieae, our molecular dataset consisted of 6.6 kb DNA (five markers) for 816 species, and yielded the first strongly supported phylogeny of the tribe (Fig. 8.S5). The Pleurothallidinae dataset was composed of 2.4 kb DNA (two markers) and 684 terminals, including, in total, 420 newly generated sequences (Fig. 8.S6). Both orchid phylogenies are strongly supported at most important nodes, with 618 nodes (76%) with BS > 75% for the Cymbidieae, and 321 nodes (47%) with BS > 75% for the Pleurothallidinae (Figs. 8.S5, 8.S6).

Ages obtained on our wide orchid-dated phylogeny were very similar to those of other recent orchid dating studies (Chomicki *et al.*, 2015; Givnish *et al.*, 2015). A chronogram for the orchid family showing absolute ages and 95% CIs for every node is provided in Fig. 8.S7. The absolute ages obtained for Cymbidieae and Pleurothallidinae chronograms are also in agreement with previously published dated phylogenies (e.g. Ramírez *et al.*, 2011; Chomicki *et al.*, 2015; Givnish *et al.*, 2016). Divergence time estimates and 95% CIs inferred for all nodes of Cymbidieae and Pleurothallidinae chronograms are shown in Figs. 8.S8 and 8.S9.

Our dating and biogeographical analyses identified the Dispersal– Extinction–Cladogenesis model with founder speciation event (DEC + J) as the best fitting model for both Cymbidieae and Pleurothallidinae (Table s S3, S4). Under this model, an Australasian origin of the Cymbidieae around the Eocene–Oligocene boundary (34 8Ma) was inferred (Figs. 8.1, 8.S8, 8.S10). We inferred a late Oligocene dispersal from Australasia to South America following the estimation of southern South America as the ancestral area of *Cyrtopodium* and the rest of the Cymbidieae (Figs. 8.1, 8.S10). Such dispersal corresponds to the final break-up of Gondwana (split between Antarctica and South America at Drake Passage). From the late Oligocene to the early Miocene, our analyses indicate dispersal from east to west in the Neotropics. The Northern Andean region was reached four times from Amazonia by MRCAs nested in Oncidiinae c. 19± 5Ma, Maxillariinae c. 11±5Ma, Stanhopeinae c. 13± 4Ma and Zygopetalinae c. 5±2Ma.

Ancestral state estimations of mean altitude further show that the MRCA of Cymbidieae was probably adapted to lowland environments (ancestral elevation value of c. 900 m; Figs. 8.S11, 8.S12). Three of the MRCAs of Amazonian migrants that reached the Andes (i.e. nested in Maxillariinae, Stanhopeinae and Zygopetalinae) were not pre-adapted to montane habitats (mean elevation values of c. 1050, 900 and 1000 m, respectively (< 1000–1100 to 2400 m; Cuatrecasas, 1958; Rangel-Churio *et al.*, 1997; Figs. 8.S11, S12). The MRCA of Oncidiinae that reached the Northern Andes, by contrast, was probably adapted to montane habitats (c. 1200 m). Strikingly, Oncidiinae and Maxillarinae are the species-richest lineages in Cymbidieae (1584 and 819 species, respectively; (Chase et al., 2015)), and are derived from both lowland Amazonian and

montane pre-adapted migrants. Stanhopeinae subsequently dispersed to several other Neotropical regions, particularly Central America (Figs. 8.1, 8.S10).

Different from the Cymbidieae, we infer an origin of Pleurothallidinae in Central America or the West Indies in the early Miocene, followed by a migration to the Northern Andes c. 16.5 Ma (Figs. 8.2, 8.S9, 8.S13), before the main uplift periods, but within a timeframe in which the Northern Andes had already achieved peak mean elevations of c. 1500 m. However, the majority of early divergent Pleurothallidinae and their sister groups are from the Antilles, and thus the inference of Central America as the ancestral area of Pleurothallidinae most probably reflects our inability to sample extensively the early diverging Antillean lineages. As inferred by ancestral state estimations, the MRCA of Pleurothallidinae was probably adapted to montane habitats (mean elevation of c. 1200 m), and all Pleurothallidinae migrants to the Northern Andes were probably adapted to montane–cloud forest environments (mean elevation of c. 1200–1300 m; Figs. 8.S14, 8.S15). BSM indicates that *in situ* speciation was the dominant biogeographical process in both clades, whereas processes of range expansion (dispersal and vicariance) and range contraction (subset speciation) were scarcer and relatively evenly distributed across lineages (Figs. 8.1, 8.2, 8.S16, 8.S17).

## 8.3.2 Diversification of Andean orchids

The diversification analyses performed with BAMM strongly rejected a constant-rate model (Bayes factor = 151.3, Table 8.S5) and, instead, identified four rate shifts during the evolutionary history of Cymbidieae (Figs. 8.3b, 8.S18, 8.S19). The best model configuration identified four shifts in speciation rate in the most speciose Cymbidieae lineages: one in Maxillariinae, one in Zygopetalinae and two in Oncidiinae.We further identified three rate shifts in the Pleurothallidinae (Table 8.S6): at the MRCA of *Lepanthes* + *Lepanthopsis*, MRCA of *Dracula* + *Porroglossum* + *Masdevallia*, and MRCA of *Stelis* + *Pabstiella* + *Pleurothallis* (Figs. 8.4b, 8.S20, 8.S21). All shifts in diversification rates in Cymbidieae and Pleurothallidinae were further confirmed using the RPANDA method (Figs. 8.S22, 8.S23; Tables 8.S7, 8.S8).

The diversification rate shifts are all located at clades that already inhabited the Northern Andes, and temporally match with periods of accelerated Andean uplift in this region (Cymbidieae, Fig. 8.1; Pleurothallidinae Fig. 8.2). To further explore this apparent correlation with either accelerated Andean uplift or presence in the Northern Andes and fast diversification, we used a trait dependent approach (GeoSSE) that estimates region-dependent speciation rates. Here, a model with free rates fitted best our Cymbidieae and Pleurothallidinae datasets (Table 8.S9), indicating significant differences in speciation (sA sB) and diversification (dA dB) rates highly if not maximally supported (0.99 and 1 Bayesian posterior probabilities, respectively). GeoSSE analyses further indicated that speciation rates in Northern Andes are consistently higher than in any other biogeographical region (Figs. 8.3c, 8.4c) in both Cymbidieae and Pleurothallidinae datasets. We evaluated and confirmed the robustness of these results through extensive data simulations (Fig. 8.S24). Here, the null distribution of GeoSSE ΔAIC values obtained from analyses with reshuffled area states was centred towards values of −20 000 and far away from the ΔAIC values obtained under analyses with real area states. We developed a novel method to generate a ‘speciation rate map’ using inferred speciation rates for each orchid lineage and georeferenced

species occurrences (see the Materials and Methods section). Our speciation rate maps are in agreement with GeoSSE results, and we confirmed that speciation rates in the Northern Andes were significantly higher than those in any other region (Figs. 8.3c, 8.4c). This is in agreement with a recent study with more limited taxon sampling for the two clades focused on here (Givnish et al., 2015). The speciation rate map (see the Materials and Methods section) further demonstrates that fastest speciation took place in the Northern Andes region, and reveals secondary speciation hotspots in the Central Andes, the Guiana Shield and Central America (Figs. 8.3d, 8.4d). These secondary hotspots are occupied by species derived from the four highly diversifying Northern Andean Cymbidieae clades (Fig. 8.S25), suggesting that the Andes acted as a major source of new lineages to the rest of the continent, thus greatly increasing Neotropical orchid diversity. This is particularly true for the Pleurothallidinae, where we identified multiple migrations from the Northern Andes of montane-adapted lineages to Central America (Figs. 8.2, 8.S26). We also found a strong geographical correlation between current species richness and diversification (Figs. 8.3d, 8.4d, 8.S27, 8.S28), suggesting that recent *in situ* speciation was the main process for species accumulation in the Neotropics.

Although these results suggest an impact of the Andean uplift on species diversification, they do not explicitly account for biotic interactions, landscape and climatic changes through time. We therefore assessed the fit of a model that explicitly integrates palaeo-elevation in diversification rate analyses (see the Materials and Methods section). In three of the four Cymbidieae clades in which BAMM inferred a speciation rate shift, the palaeo-elevation-dependent model inferred a continuous speciation increase from 10 to 6 Ma as a result of a positive correlation between speciation and palaeo-elevation (Fig. 8.3e,f; Table S10). By contrast, no positive correlation with palaeo-elevation and diversification could be detected for Pleurothallidinae (Table 8.S11). Moreover, our ancestral character estimation of pollination syndromes in Cymbidieae suggests that the MRCA of Cymbidieae was bee pollinated (Fig. 8.S29). Nine shifts of syndromes were identified along the evolutionary history of Cymbidieae, always derived from bee pollination. No reversals from other syndromes towards bee pollination were recovered (Fig. 8.S29).

# 8.4 Discussion

## 8.4.1 Andean orchids are derived from lowland Amazonian, montane Central American and local sub-Andean migrants

Our ancestral area estimations show that Andean orchid flora is derived primarily from Amazonian lowland taxa (i.e. MRCAs of Andean clades of Maxillariinae, Stanhopeinae and Zygopetalinae, from which most of the species-richest lineages in Cymbidieae originated), but also from cool pre-adapted lineages (MRCAs of both Andean Oncidiinae and most extant Andean-centred pleurothallid taxa). Previous research has revealed that mountain flora origin is strongly influenced by the immigration of cool preadapted lineages (Hughes and Eastwood, 2006; Merckx *et al.*, 2015; Uribe-Convers and Tank, 2015), and that contributions from lowland-adapted lineages is rather rare. In Borneo, a large portion of the mountain endemics of Mount Kinabalu arose from pre-adapted lineages from other cool areas (Merckx et al., 2015), but *Dendrochilum* orchid montane endemics arose from low-elevation local ancestors (Barkman and Simpson,

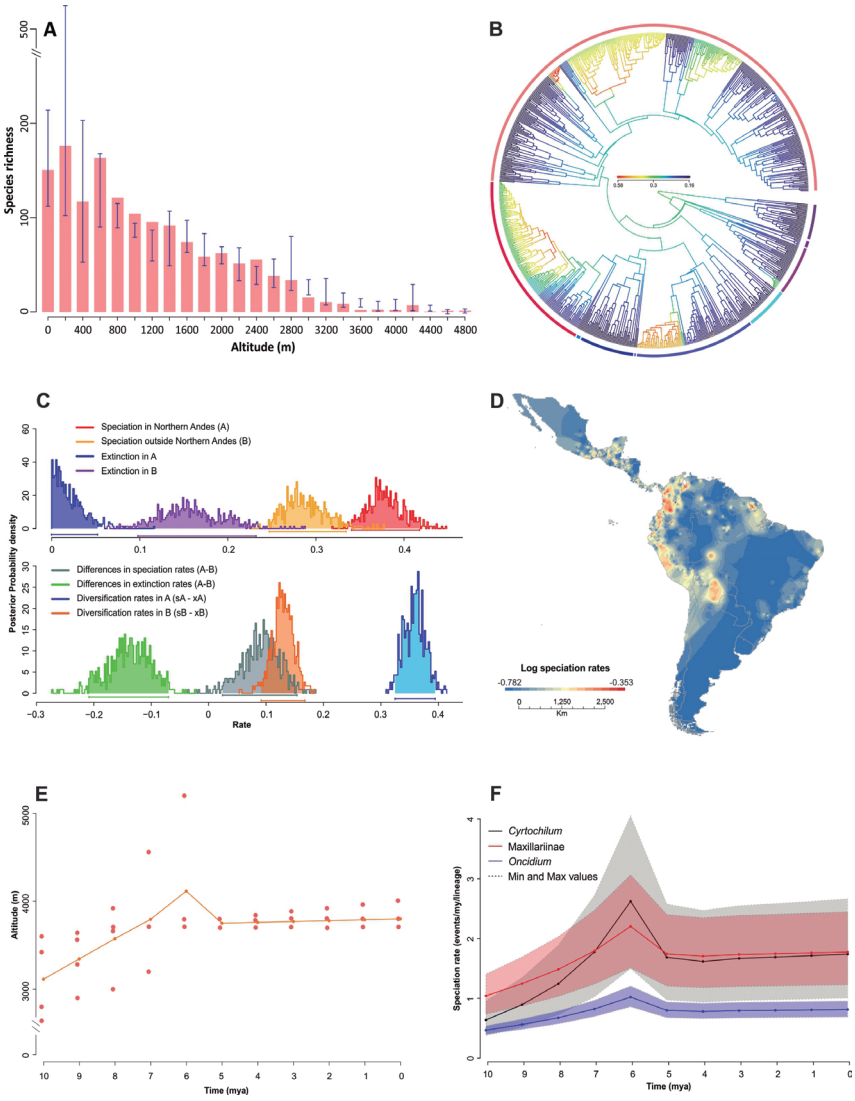


**Figure 8.3.** Diversification of the Cymbidieae. **A.** Richness vs elevation plot for 55%(> 20 000 herbarium records) of the c. 4,000 Cymbidieae species. Blue error bars indicate maximum and minimum species richness values. **B.** Speciation rate plot (phylorate) showing the best configuration shift identified by BAMM. Colour intensity across branches is proportional to changes in diversification rates. (c) Density probability plots of speciation, extinction and net diversification rates per area identified by GEOSSE. Area 'A' refers to species restricted to the Northern Andes; area 'B' refers to species occurring in all areas except the Northern Andes. **D.** Speciation ratemap estimated fromBAMM (see the Materials and Methods section). **E.** Average palaeo-elevation of the Central and Northern Andes. **F.** Palaeo-elevation-dependent models applied to the four clades detected by BAMM to have significantly higher diversification rates than others. Lineages in (B) are colour coded in the same way as shown in Fig. 8.1. Timescale in panels (E) and (F) is expressed in million years ago (Ma).

2001). Similarly, epiphytic, tuberous Rubiaceae (Hydnophytinae) endemics from New Guinea montane habitats originated from local lowland migrants (Chomicki and Renner, 2017). Our study points to the key role of Amazonia for the origin of Andean orchid diversity, and also reveals an ancient biological connectivity between Amazonia and the Northern Andes.

## 8.4.2 The Andes did not constrain orchid dispersal

The recurrent migration back and forth through the Andes, even during the period of highest palaeo-elevation, is also a central result from our study. The colonization of the Northern Andes by some clades of Cymbidieae matches in time with accelerated surface uplift (Figs. 8.1, 8.S10), and reflects the Miocene biotic connectivity between the Andes and Amazonia previously suggested for plants (Antonelli et al., 2009a), Poison dart frogs (Santos et al., 2009), and birds (Brumfield and Edwards, 2007), among others. This suggests that shifts across elevational zones were not rare, contrary to recent results in Mount Kinabalu in Borneo (Merckx et al., 2015).

Surprisingly, dispersal events across the Andes did not decrease during accelerated Andean uplift (Figs. 8.1, 8.2, 8.S10, 8.S13), suggesting that the uplift of the Andes did not act as a major dispersal barrier for Cymbidieae and Pleurothallidinae orchids, contrary to findings in other plant groups (e.g. Annonaceae, (Pirie et al., 2006); Rubiaceae, (Antonelli et al., 2009b); or Fabaceae, (Pennington et al., 2010). This result probably relates to the biology of orchids, which produce large amounts of dust-like, wind-dispersed seeds, allowing for occasional long-distance dispersal (Antonelli et al., 2009a; Arditti and Ghani, 2000; Barthlott et al., 2014; Givnish et al., 2016; Pérez-Escobar et al., 2017), enabling occasional crossing of the Andes, and perhaps more frequently migration to different elevation zones. Taken together, these findings suggest that the Andes constitutes a semipermeable barrier to biotic dispersal, and that orchids may be more geographically constrained by intrinsic factors, such as fungal symbionts and pollinator mutualists, which differ among elevational zones (Arroyo et al., 1985, 1982; Lugo et al., 2008) than by distance. The dependence of immigrant orchids on particular fungal or pollinator mutualists, matched to the available pool of mutualists, may greatly determine the success of their establishment in a new area. Our findings of widespread within-region speciation as the main biogeographical process (Figs. 8.1, 8.2, 8.S16, 8.S17), coupled with the apparent widespread permeability of the Andean mountains to lowland migrants, raise the question of the speciation mechanisms underlying these fast speciation rates. We speculate that the habitat heterogeneity, with many adjacent but distinct niches, could have favoured isolation, perhaps via peripatric or parapatric speciation. Be as it may, our work paves the way for microevolutionary studies of orchid speciation in the Andes.

## 8.4.3 Accelerated orchid diversification across elevational zones

Gentry's hypothesis of rapid speciation (Gentry, 1982) in the Andes was mainly based on the observation of floristic groups (e.g. 'Andean-centred taxa') with very speciose genera from the lowlands to mid-elevations in the (mostly Northern) Andes. This matches well the total altitudinal distribution of our respective study groups, with a richness vs elevation plot for > 55% of the 3,700 Cymbidieae species based on over 20,000 records (Figs. 8.3a, 8.S1), which reveals that

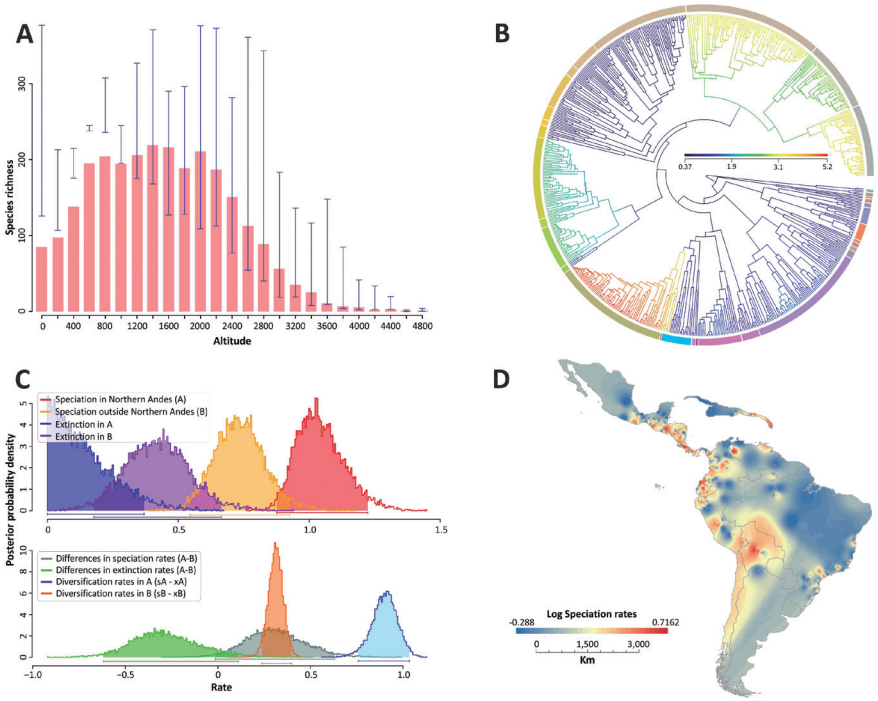


**Figure 8.4.** Diversification of the Pleurothallidinae. **A.** Richness vs elevation plot for 50% (> 9000 herbarium records) of the c. 5000 Pleurothallidinae species. Blue error bars indicate maximum and minimum species richness values. **B.** Speciation rate plot (phylorate) showing the best configuration shift identified by BAMM. Colour intensity across branches is proportional to changes in diversification rates. **C.** Density probability plots of speciation, extinction and net diversification rates per area identified by GEOSSE. Area 'A' refers to species restricted to the Northern Andes; area 'B' refers to species occurring in all areas except the Northern Andes. **D.** Speciation rate map estimated from BAMM (see the Materials and Methods section). Lineages in (B) are colour coded in the same way as shown in Fig. 8.2.

Cymbidieae diversity peaks at low elevations (< 1,100 m), whereas Pleurothallidinae diversity (c. 10,000 records; Fig. 8.S2) peaks at c. 1,500 m (Fig. 8.4a).

The diversification rate shifts are all located within clades that already inhabited the Northern Andes, and temporally match with periods of accelerated Andean uplift in this region (Gregory-Wodzicki, 2000; Hoorn et al., 2010) (Figs. 8.1, 8.2). The late middle Miocene and early Pliocene are the periods with the fastest documented rates of Andean uplift in the Northern Andes (i.e. Venezuelan Andes and Northern Andes of Colombia; Hoorn *et al.*, 1995; Bermúdez *et al.*, 2015). In all three Cymbidieae clades, speciation rates peaked at 6 Ma, a time at which the Northern Andes reached c. 4,000 m, their maximum mean palaeo-elevation (Bermúdez et al., 2015). Contrary to Cymbidieae, we found no correlation between Andean uplift and Pleurothallidinae diversification (Table 8.S11). We hypothesize that this is a result of the rapid diversification of migrating cool preadapted Pleurothallidinae lineages from Central America into already formed

montane environments (Hoorn et al., 2010). Similar diversification patterns have been reported for *Lupinus*, *Bartsia*, Adoxaceae, Valerianaceae and, more recently, Ericaceae (Donoghue and Sanderson, 2015; Schwery et al., 2015; Uribe-Convers and Tank, 2015).

Gentry proposed that the main mechanism underlying rapid speciation in the Andes was the evolution of novel plant–insect interactions (Gentry, 1982). The Cymbidieae are particularly known among biologists and ecologists because of the rich array of pollination syndromes and sexual systems they have evolved (e.g. sexual and food deceit, food and fragrance reward, dichogamy and environmental sex determination; Gerlach and Schill, 1991; Singer, 2002; Pansarin *et al.*, 2009; Gerlach and Pérez-Escobar, 2014). Our analyses suggest that pollinator syndrome shifts do not match with diversification rate shifts, although our data do not take into account pollinator shifts within given pollinator groups. This is particularly true for the bee pollination syndrome, which is widespread in the tribe and probably overarches several transitions from different types of bees (e.g. oil to euglossine bees as observed in Catasetinae). More field observations of pollinations are therefore needed to evaluate the relative role of pollinator shifts in contributing to Neotropical orchid diversification.

## 8.5 Conclusions

Based on two extensively sampled orchid phylogenies, combined with statistically robust diversification models, our results reveal that Andean orchid diversification has closely tracked the Andean orogeny. Together with studies in other mega-diverse regions (Bruyn et al., 2014; Verboom et al., 2009), our results show that rapid recent speciation has moulded this area of exceptional species richness. In addition, our results highlight the crucial role of Amazonian lowlands, as well as the Antillean and Central American regions, as biotic sources for Andean biodiversity, providing cool pre-adapted lineages that dispersed into the Andes and further diversified *in situ*.

Contrary to general expectation, the rise of the Andes had little effect on restricting orchid biotic dispersal across the Neotropics. This suggests that mountains are semi-permeable barriers to lowland organisms, whose dispersal ability is more probably related to intrinsic traits (e.g. seed size, dispersal mechanism, mutualisms). Although both abiotic and biotic processes are clearly responsible for the exceptional species richness of the world's premier biodiversity hotspot (Antonelli and Sanmartín, 2011; Hughes *et al.*, 2013; Eiserhardt *et al.*, 2017), our results suggest that geological processes played a central and direct role in the diversification process. Finally, as the highest species richness in Cymbidieae is concentrated in the lowlands and the Pleurothallidinae peak is at mid-elevation, our study shows that Andean uplift dramatically affected the evolutionary assembly of both lowland and mid-elevation Andean forests, as originally hypothesized by Gentry (1982).

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