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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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# Chapter 9

## Speciation and biogeography of the hyperdiverse genus *Lepanthes* (Orchidaceae: Pleurothallidinae)

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*To be submitted*

**Abstract.** *Lepanthes* is one of the six most species-rich angiosperm genera in the Neotropics accounting for more than 1,130 species. The influence of extrinsic factors on the diversification of the genus was assessed in a broad-scale analysis in Chapter 8. Here, we used *Lepanthes* as a model to understand patterns of diversification in the Neotropics driven by intrinsic factors. We inferred the biogeographical history of the genus based on a time-calibrated chronogram obtained by a molecular phylogenetic analysis with a species sampling increased up to 25%. Our results show that *Lepanthes* likely originated in the Central Andes (CA) and diversified between 7–8 Ma. The genus reached Southern Central America (SCA) from the Andean region twice, with one recolonization to the Northern Andes (NA) from SCA. The extant lineages from Northern Central America (NCA) and the West Indies (WI) are likely derived from SCA ancestors. Cladogenesis by within-area speciation was the most common biogeographical event and the most frequent dispersal routes were SCA with NCA and NA and NA with CA. *Lepanthes* showed the highest rates of species diversification in the Pleurothallidinae and two of its most recent clades from SCA experienced shifts in species diversification with an acceleration around 2.5 Ma. This acceleration did not strictly correlate with mountain orogeny as found in Chapter 8 for the Epidendroideae as a whole. However, paleoclimatic evidence indicates that cooling periods started before 2.7 Ma and this partially correlates with *Lepanthes* diversifications in SCA. Although species sampling from SCA was intensive, the sampling of Andean, NCA and WI lineages was still low and should be increased. Likewise, more accurate species distribution data and alpha-taxonomical expertise are needed to obtain more insight in the most important intrinsic factors driving speciation and biogeography of *Lepanthes*.

## 9.1 Introduction

*Lepanthes* Sw. is one of six most species-rich angiosperm genera in the Neotropics accounting for more than 1130 species (Bogarín et al., 2018c). *Lepanthes* and closest allies are widely distributed from Mexico and Florida to southern Brazil and Argentina, including Central America and the Antilles (Luer and Thoele, 2012). Most of the species are concentrated in Costa Rica-Panama (160 spp.), Colombia-Ecuador (>300 spp. each) and Peru-Bolivia (>100 spp.). Multiple hypotheses exist about the factors that drove this extraordinary diversity. These include intrinsic characters such as trait evolution (Chapter 2), pollinator specialization (Chapter 6, Blanco and Barboza, 2005) and extrinsic traits such as colonization, orogeny or climatic fluctuations (Chapter 8, Givnish et al., 2015, 2016; Pérez-Escobar, Chomicki, et al., 2017). The influence of extrinsic factors on the diversification of the most speciose Neotropical orchid lineages (Pleurothallidinae and Cymbidiae) was assessed in Chapter 8 (Pérez-Escobar et al., 2017a). These authors discovered that rapid recent speciation predominates in the most speciose lineages such as *Lepanthes* and that the rise of mountain ranges had little effect on constraining orchid dispersal. This suggests that mountains are semi-permeable barriers and dispersal restriction is more related to intrinsic traits. In addition, these authors found that Central America has been an important biotic source for Andean biodiversity, providing cool pre-adapted lineages that dispersed into the Andes and further diversified. In Chapter 8 we addressed those biogeographical hypotheses on a broad scale by taking the entire Pleurothallidinae as a model. As a continuation of their study, we used *Lepanthes* as a model to further understand its patterns of diversification in the Neotropics. To achieve this, we inferred the biogeographical history of *Lepanthes* by increasing taxon sampling, including both key representatives of main clades and main biogeographical areas such as Northern Central America and the West Indies, and producing a time-calibrated chronogram based on nuclear nrITS and plastid *matK* markers covering about 25% of all species of *Lepanthes* and close allies (Bogarín et al., 2018c). This chapter discusses the ancestral range of *Lepanthes*, the most likely colonization routes across the Neotropics, the most common biogeographical models and its diversification rates through time. The combined results show that *Lepanthes* underwent rapid diversification and dispersed across the Neotropics during a series of climatological changes and *in situ* speciation events.

## 9.2 Materials and Methods

### 9.2.1 Taxon sampling

We sequenced the nuclear ribosomal internal transcribed spacer (nrITS) and the plastid maturase K (*matK*) of 351 accessions of *Lepanthes*. In addition, we included previously generated sequences of the 13 genera related to *Lepanthes* from Chapters 2-3 and 8. About 20% of the species of *Lepanthes* were sampled across the Neotropics with emphasis on Southern Central America. Voucher information, NCBI GenBank accessions, and references for each DNA sequence are listed in Table 9.1. *Acianthera butcheri* (L.O.Williams) Pridgeon & M.W.Chase and *Acianthera fenestrata* (Barb.Rodr.) Pridgeon & M.W.Chase were chosen as outgroups based on Pridgeon et al., (2001).

### 9.2.2 DNA extraction, amplification, sequencing and alignment

We obtained total genomic DNA from about 50-100 mg of silica gel dried leaf/flower tissue powdered in a Retsch MM 300 shaker. We followed the 2× CTAB (Hexadecyltrimethylammonium bromide) protocol for isolating DNA (Doyle and Doyle, 1987). The polymerase chain reaction (PCR) mixture, the primers for the nrITS (17SE and 26SE) and plastid *matK* (2.1aF and 5R) regions and amplification profiles are described in Chapters 2 and 8. Sanger sequencing of both regions was conducted by BaseClear (<https://www.baseclear.com>) on an ABI 3730xl genetic analyzer (Applied Biosystems, Foster City, California, U.S.A). Sequences were deposited in NCBI GenBank (Table 9.1). We used Geneious® R9 (Biomatters Ltd., Auckland, New Zealand (Kearse et al., 2012)) for the editing of chromatograms and pairwise alignment. Sequences were aligned in the online MAFFT platform (Multiple Alignment using Fast Fourier Transform, <http://mafft.cbrc.jp/alignment/server/>) using default settings. We adjusted and trimmed the resulting alignment manually.

### 9.2.3 Phylogenetic analyses and divergence time estimation

We obtained gene trees for each individual nrITS and *matK* dataset with maximum likelihood (ML) in RAxML-HP2 on XSEDE (8.2.10) (Stamatakis et al., 2008) choosing the GTRGAMMA model for bootstrapping and 1,000 bootstrap iterations. For each dataset, the model of evolution was calculated with the Akaike Information Criterion (AIC) in jModelTest2 v2.1.7 (Darriba et al., 2012). To evaluate the incongruence between nrITS (nuclear) and *matK* (plastid) datasets we followed the pipeline implemented by Pérez-Escobar, Balbuena, and Gottschling (2016) using the Procrustean Approach to Cophylogeny (PACo) application (Balbuena et al., 2013) in R (<http://data-dryad.org/review?doi=doi:10.5061/dryad.q6s1f>). The conflicting terminals were excluded from the *matK* dataset and replaced by missing data. The resulting *matK* dataset was further concatenated with the nrITS dataset in Sequence Matrix v100.0 (Vaidya et al., 2011). This concatenated dataset was used to estimate the divergence times in BEAST v.1.8.2. In addition, the statistical support of the clades was evaluated with the values of posterior probability (PP) for the Bayesian Inference reconstruction. We performed two MCMC with  $60 \times 10^6$  generations and sampling every 1,000 generations with a Marginal likelihood estimation (MLE) of 50 path steps,  $10 \times 10^5$  length of chains and log likelihood for every 1,000 generations. The clock-likeness of the data was tested with the coefficient of variation (CV) of relaxed clock models. Speciation tree model selection was achieved by executing the Bayes factor test using the MLE from the stepping stone sampling on Yule Process (Y), Birth Death-Process (BD) and Birth-Death-Incomplete Sampling (BDIS) models under strict and uncorrelated lognormal molecular clock models. For each model, we assigned a normal prior distribution of 16.45 Ma and 2.5 SD (standard deviations) to the root node, 12.93 Ma and 2.5 SD to the node of the MRCA of the *Lepanthes* clade and 12.93 Ma and 2 SD to the MRCA of *Zootrophion* and the remainder of the members of the *Lepanthes* clade. These secondary calibrations were calculated from the values obtained from the time-calibrated chronogram of the Pleurothallidinae by Pérez-Escobar et al., (2017b) (Chapter 8). We inspected the convergence of independent runs size and the MCMC stationarity of parameters (ESS values >200) in Tracer v.1.6. A maximum clade credibility (MCC) tree was obtained with a 10% of burnin using TreeAnnotator v.1.8.2. All phylogenetic analyses and dating

analyses were run in the CIPRES Science Gateway V. 3.1 ([http://www.phylo.org/sub\\_sections/portal/](http://www.phylo.org/sub_sections/portal/)) (Miller et al., 2010). Resulting trees and the 95% highest posterior density (HPD) estimations were viewed in FigTree v1.4.3 (Rambaut, 2006) and manipulated with R programming language (R Core Team, 2017) under R Studio (Gandrud, 2013) using the packages APE, ggtree and phytools (Paradis et al., 2004; Revell, 2012; Yu et al., 2017). Final trees were edited in Adobe® Illustrator CC (Adobe Systems Inc., California, U.S.A).

### 9.2.4 Ancestral range estimation (ARE)

For the range estimations we obtained geographical records from herbaria (AMES, CR, JBL, K, L, SEL, US, W), online databases such as TROPICOS (<http://www.tropicos.org>), WCSP (<https://wcsp.science.kew.org/>), Epidendra ([www.epidendra.org](http://www.epidendra.org)) and the Global Biodiversity Information Facility GBIF (<https://www.gbif.org>). This information was used to encode the accessions of the concatenated dataset in eight main distribution areas according to the current distribution of the genus: Northern Central America (NCA) (comprising southern Mexico to Nicaragua); Southern Central America (SCA) (comprising Costa Rica and Panama) in addition to West Indies (WI); Northern Andes (NA); Central Andes (CA); Amazonia and Guiana Shield (A); South-eastern South America (SSA) and Chocó (Ch) as defined in Chapter 8. This range matrix and the MCC tree obtained from the BEAST dating analysis were used to infer the ancestral range of *Lepanthes* and allied genera with the R-package BioGeoBEARS v.0.2.1. BioGeoBEARS calculates probabilistic inferences of ancestral geographic ranges on a phylogeny and allows model fit selection with statistical tests (Matzke, 20018). Therefore, we evaluated six models: (1) dispersal–extinction–cladogenesis, DEC (implemented in LAGRANGE, Ree and Smith, 2008), (2) DEC+J, allowing founder-event speciation, (3) DIVALIKE, a ML version of dispersal–vicariance analysis (DIVA) (Ronquist, 1997), and (4) DIVALIKE+J, allowing founder-event speciation, (5) BAYAREALIKE, the ML version of Bayesian inference of historical biogeography (BAYAREA) (Landis et al., 2013) and (6) BAYAREALIKE+J, allowing founder-event speciation. We selected the best model-fit with the weighted AIC and likelihood ratio test (LRT) scores calculated in BioGeoBEARS (Matzke, 2014, 2013). In addition, to infer biogeographical events such as migrations, dispersals, extinctions and within-area speciations we implemented the biogeographical stochastic mapping (BSM) approach generating 50 stochastic maps in BioGeoBEARS (Matzke, 2014, 2013).

### 9.2.5 Rates of species diversification

To infer the diversification dynamics (extinction and speciation rates) we used a time-dependent model implemented in the C++ program BAMM v.2.5.0 (Bayesian Analysis of Macroevolutionary Mixtures) (Rabosky et al., 2014a). In this analysis, we assigned a sampling fraction of 25% of the extant diversity of the *Lepanthes* clade. We performed four runs with  $5 \times 10^6$  Markov chain Monte Carlo (MCMC) generations, sampling parameters every 1,000 generations. We checked the convergence of the runs by plotting the log-likelihood across MCMC generations. The diversification rates and rate shifts calculated with BAMM were analyzed and plotted with the R-package BAMMtools v2.1.6 (Rabosky et al., 2014b). In addition, we selected the best

model with a Bayes Factors test and examined the 95% credible set of macroevolutionary shift configurations with the functions *ComputeBayesFactor* and *CredibleShiftSet* of BAMMtools. Recently, reliability BAMM has been questioned due to theoretical issues of the likelihood function and the incoherent compound Poisson process prior model (Moore et al., 2016). However, Rabosky et al., (2017) demonstrated that inferences about diversification rates have been accurate with the BAMM software and that diversification rates can be inferred using several methods such as BAMM.

## 9.3 Results and discussion

### 9.3.1 Phylogenetic relationships of *Lepanthes* and allied genera

A total of 132 terminals out of 300 of the *matK* dataset were detected as incongruent. The best speciation tree model obtained with the Bayes Factors test was the Yule Process, therefore the MCC tree obtained from this analysis was used to infer the divergence dates, ancestral range estimation and rates of species diversification. The support values slightly increased after removing the potential outliers from the plastid dataset and the main clades received strong support in the BEAST analyses. The monophyly of *Anathallis*, *Draconanthes*, *Lepanthes*, *Pendusalpinx*, *Stellamaris*, *Trichosalpinx* s.s., *Tubella* and *Zootrophion* (all with PP=1.0), *Lepanthopsis* (PP=0.99) and *Lankesteriana* (PP=0.96) was highly supported. Although *Gravendeelia*, *Fronдаря* and *Opilionanthe* were recognized as distinct lineages their phylogenetic relationships remain unresolved with the molecular markers currently analyzed. The intergeneric relationships are congruent with previous phylogenies of the group though (Bogarín et al., 2018c) (see Chapters 4 and 5). After increasing species sampling, *Lepanthes* was again recovered as a monophyletic group (PP=1.0).

### 9.3.2 Divergence times and historical biogeography

The DEC+J model was significantly better than the DEC model according to the LRT ( $p < .00001$ ) and received the highest likelihood score (AIC=935.4) among the six models tested (Table 9.2). The most recent common ancestor (MRCA) of *Lepanthes* and allied genera was estimated to have evolved around 13.14 Ma (95% HPD: st.dev. 10.42-15.87 Ma) during the Miocene. Most of the extant taxa diverged in the Miocene-Pliocene with MRCAs estimated for *Zootrophion* to have evolved around 3.91 Ma (st.dev. 2.07-6.58 Ma), *Anathallis* 6.87 Ma (st.dev. 4.57-9.56 Ma), *Tubella* 6.55 Ma (st.dev. 4.2-9.35 Ma), *Trichosalpinx* 7.15 Ma (st.dev. 4.48-10.25 Ma), *Lankesteriana* 6.55 Ma (st.dev. 5.62-10.83 Ma), *Lepanthopsis* 4.64 Ma (st.dev. 2.73-6.90 Ma), *Stellamaris* 9.49 (st.dev. 7.23-12.03 Ma) and *Pseudolepanthes* 8.51 Ma (st.dev. 6.43-10.08 Ma). Unfortunately, most of the ancestral ranges of these groups remained unresolved (Fig. 9.1). The ancestral ranges of *Trichosalpinx* and *Tubella* were estimated as Southern Central America, however, more sampling from other geographical regions is needed to confirm this inference because our sampling lacked key representatives from other regions (See Chapter 5). In addition, *Draconanthes* and *Lepanthes* diverged at around 8 Ma (st.dev. 6.0-10.12 Ma) in the Andean region (marginal probabilities for each range: E=0.48; F=0.12; EF=0.31, see Fig.

**Table 9.2.** Comparison of the six biogeographical models implemented in BioGeoBEARS fitted on the *Lepanthes* dataset with the likelihood ratio ( $\chi^2$ ) and AIC test evaluating null models against the alternative model with the founder speciation event “J” parameter. Best fitting model indicated in **boldface**.

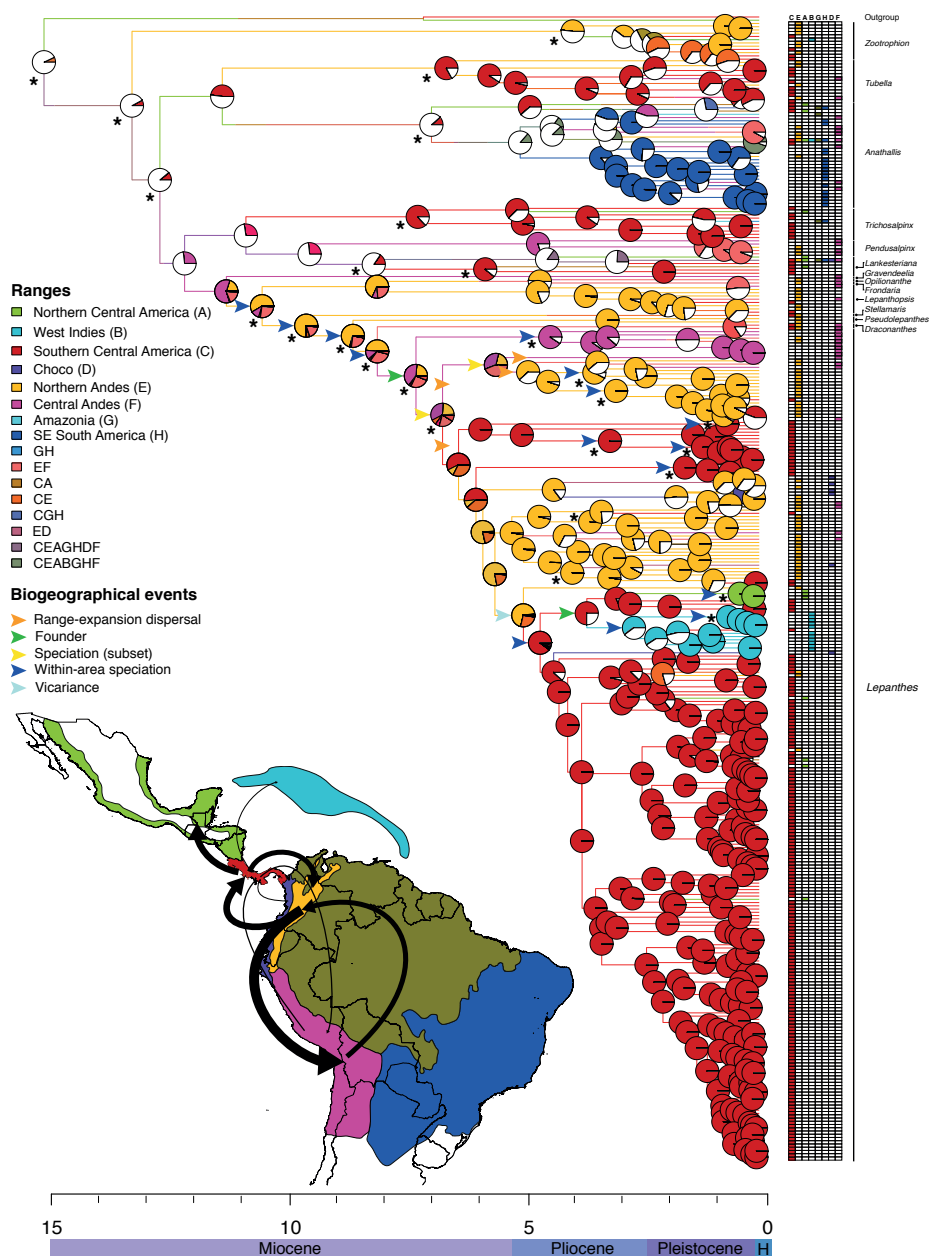
Alt. <sup>1</sup> model	Null model	LnL Alt	LnL null	Dstatis- tic	pval	AIC Alt.	AIC Null	AICwt <sup>2</sup> Alt.	AICwt Null	AICwt ratio Alt.	AICwt ratio Null
<b>DEC+J</b>	DEC	-464.7	-488.9	48.29	3.70E-12	935.4	981.7	1	8.90E-11	1.13E+10	8.90E-11
DIVALIKE+J	DIVALIKE	-488.1	-503.6	30.86	2.80E-08	982.2	1011	1	5.40E-07	1844349	5.40E-07
BAYAREALIKE+J	BAYAREALIKE	-483.9	-574.2	180.7	3.50E-41	973.8	1152	1	1.60E-39	6.26E+38	1.60E-39

**Table 9.3.** Results from 50 BSM under the DEC+J model in BioGeoBEARS. Mean of all biogeographical events across the 50 BMSs among areas (coded by letters) for the *Lepanthes* and allied genera.

		To									
From	C	E	A	B	G	H	D	F			
	0.00	7.3 (1.45)	9.58 (1.14)	1.42 (0.57)	1.68 (0.74)	1.54 (0.81)	1.16 (0.55)	2.64 (1.19)			
	E	9.62 (1.92)	0.00	0.32 (0.55)	0.84 (0.65)	0.2 (0.4)	0.28 (0.57)	3.84 (0.58)	14.42 (2.34)		
	A	1.04 (0.95)	0.22 (0.42)	0.00	0.12 (0.33)	0.3 (0.51)	0.22 (0.42)	0.12 (0.33)	0.24 (0.48)		
	B	0.86 (0.81)	0.14 (0.35)	0.08 (0.27)	0.00	0.1 (0.3)	0.1 (0.3)	0 (0)	0.24 (0.43)		
	G	0.12 (0.33)	0.1 (0.3)	0.2 (0.4)	0.04 (0.2)	0.00	1.22 (1)	0.2 (0.4)	0.56 (0.58)		
	H	0.2 (0.57)	1.24 (0.52)	0.2 (0.4)	0.04 (0.2)	1.22 (0.86)	0.00	0.14 (0.35)	3.34 (0.66)		
	D	0.12 (0.44)	0.4 (0.73)	0.08 (0.27)	0 ( )	0.1 (0.3)	0.1 (0.3)	0.00	0.1 (0.3)		
	F	1.88 (1.22)	7.3 (2.3)	0.44 (0.64)	0.08 (0.27)	0.7 (0.61)	0.88 (0.9)	0.16 (0.37)	0.00		



9.1 for the coding of areas) and *Lepanthes* originated around the end of the Miocene, around 7.2 Ma (st.dev. 5.4–9.2 Ma) in the Andes with a slightly higher probability for the central Andes ( $E=0.22$ ;  $F=0.39$ ;  $EF=0.28$ ) (Fig. 9.1). From this *Lepanthes* ancestor, one lineage evolved in the Central Andes during the Pliocene ( $F=0.91$ ), comprising the extant species related to *L. tigrina* Luer & Thoele, *L. terborchii* Luer & Sijm, *L. nycteris* Luer & R.Vázquez, *L. caprimulgus* Luer (*L. tigrina* group) ( $PP=1.0$ ). Its sister lineage, containing the remaining species of *Lepanthes*, diverged earlier during the Miocene and likely in the Andes ( $E=0.22$ ;  $F=0.31$ ;  $EF=0.22$ ). From this ancestor, another eminently North-Central Andean clade was derived in the end of the Miocene ( $E=0.20$ ;  $F=0.33$ ;  $EF=0.47$ ) made up of species related to *L. juninensis* Schltr. (*L. juninensis* group) in addition to the first ancestor of a likely Southern Central American origin ( $E=0.09$ ;  $C=0.55$ ;  $CE=0.35$ ), which diverged earlier at around 6 Ma during the Miocene-Pliocene boundary. This ancestor yielded the first lineages that originated in Southern Central America, represented today by several clades endemic to Costa Rica and Panama ( $C=1.0$ ) such as *L. horrida* Rchb.f., *L. jimenezii* Schltr. and *L. minutilabia* Ames & C.Schweinf. This clade is sister to another clade also derived from a Southern Central American or Andean ancestor ( $E=0.09$ ;  $C=0.49$ ;  $CE=0.41$ ). Shortly after this splitting, several Northern Andean ancestors evolved between 6–5 Ma represented in a serially branching pattern along the MCC tree ( $E=>0.74$ ;  $CE=<0.24$ ), which gave rise to South American *Lepanthes* groups such as *L. calodyction* Hook., *L. felis* Luer & R. Escobar and *L. hexapus* Luer & R. Escobar and a few extant Central American and Chocoan lineages. This suggests a possible recolonization of the Andes from Central America. Around 5 Ma, another Southern Central American ancestor descended from these lineages that evolved again from a Northern Andean ancestor and diversified in the extant taxa found in Costa Rica and Panama ( $C=0.9$ ). This event represents a second colonization from an Andean ancestor in Southern Central America. In addition, the lineages from Northern Central America and the West Indies were derived from this second Southern Central American colonization in the Pliocene-Pleistocene suggesting that the isthmus of Panama served as a land bridge for lineages derived from Andean ancestors. Therefore, a colonization of the West Indies and Northern Central America from the Guyanas and Amazonia is not supported. The most frequent dispersal routes recorded were among Southern Central America with Northern Central America, Southern Central America with the Northern Andes and vice versa and the Northern Andes with the Central Andes (Fig. 9.1, Table 9.3). As found in Chapter 8, the MRCA of Pleurothallidinae was likely adapted to montane habitats, and migrants to the Northern Andes were probably adapted to montane–cloud forest environments. Therefore, the rise of the Talamanca range in Southern Central America likely benefited the colonization of preadapted montane–cloud forest lineages that eventually also colonized Northern Central America and the highlands of the West Indies (Pérez-Escobar et al., 2017a). Some lineages also colonized lowland areas during Pleistocene climatic fluctuations. In addition, a few extant lowland Chocoan lineages descended from both Andean and South Central American ancestors. The remaining species from Costa Rica and Panama diverged very recently (about 2.5 Ma) from Andean ancestors in the Pliocene-Pleistocene. The age estimations calculated here are similar to those obtained in Chapter 8 and other chronograms of the Orchidaceae but with narrower 95% HPD intervals (Chomicki et al., 2015; Givnish et al., 2016; Pérez-Escobar et al., 2017a).



**Figure 9.1.** Time-calibrated phylogenetic tree of *Lepanthes* and estimated ancestral ranges with BIOGEOBEARS under the DEC+J model. Pie charts at the nodes indicate the relative probability of each estimated ancestral area and colored branches are from one of the BSM. Colored biogeographical ranges are represented with boxes and corresponding name of the range and letter (code) assigned. The combinations of letters refer to ancestral areas made up of more than one biogeographical area. Ranges of every taxon are shown as a

**Table 9.4.** Summary of the biogeographical stochastic mapping with the DEC+J model showing event counts across 50 BSMs, mean of events and standard deviations.

Events	Mode	Type	Number of events	Mean (SD)	%
Cladogenetic	Within-area speciation	Speciation ( <i>in-situ</i> )	13751	275.02 (3.51)	68.19
		Speciation (subset)	1670	33.40 (4.57)	8.28
	Vicariance	Vicariance	755	15.10 (3.67)	3.74
	Dispersal	Founder events	1424	28.48 (4.13)	7.06
Anagenetic	Dispersal	Range-expansion dispersal (“d” parameter)	2565	51.30 (4.45)	12.72
		Range-switching dispersal (“a” parameter)	0	0	0
		Range-contraction dispersal (“e” parameter)	0	0	0
Total				403.3 (4.45)	100.00

### 9.3.3 Estimation of biogeographical events

Results of the BSM approach indicate that most biogeographical events correspond to cladogenesis by within-area speciation (68.20%) with minor contributions from speciation (subset), vicariance, founder events and anagenetic events (3-12% of the total estimates) (Fig. 9.3, Table 9.4). Among them, vicariance was the least favoured event whereas speciation (subset), founder events and range expansion showed similar contributions. The BSM approach suggested that *Lepanthes* likely originated from a founder event from the Northern Andes to Central Andes and subsequent speciation (subset) and range-expansion dispersal. Once the areas were colonized, within-area speciation was the most important event. The large contribution of within-area speciation is probably related to the high levels of endemism in *Lepanthes* (Luer and Thorerle, 2012), the large delimitation size of some of the Andean regions, which are rich in species (e.g. Central and Northern Andes) or the sampling bias to Southern Central American species. The rarity of vicariance events suggest that orchid dispersal is not constrained by the raise of Neotropical mountain ranges as hypothesized in Chapter 8 (Pérez-Escobar, Chomicki, et al., 2017b; Pérez-Escobar, Gottschling, et al., 2017). *Lepanthes* likely reached Southern Central America from the Andes at least twice, the first time via speciation (subset) event at about 6.5 Ma and the second time by vicariance at around 5 Ma. This suggests a biotic connectivity between the

heatmap after the terminals. Asterisks (\*) indicate node supports of PP > 0.95. Some biogeographical events (range-expansion dispersal, founder, speciation (subset), within-area speciation and vicariance) are shown on the nodes and branches with colored arrowheads. (Inset map) Coded areas for biogeographical analysis corresponding to the colored ranges (boxes) and most frequent dispersal routes represented by arrows proportional to the frequency of events. Political divisions obtained from DIVA-GIS (<http://www.diva-gis.org/gdata>). Timescale shown at bottom is expressed in million years ago (Ma).

Andes and SCA as also found for other extant angiosperm species of the paramo and montane areas of Andean origin (e.g. *Puya*, Bromeliaceae). Although within-area speciation was the main biogeographical event, founder and vicariance were important events for colonizing new areas in *Lepanthes*. Particularly, founder events were important in the colonization of Northern Central America and the West Indies from Southern Central America.

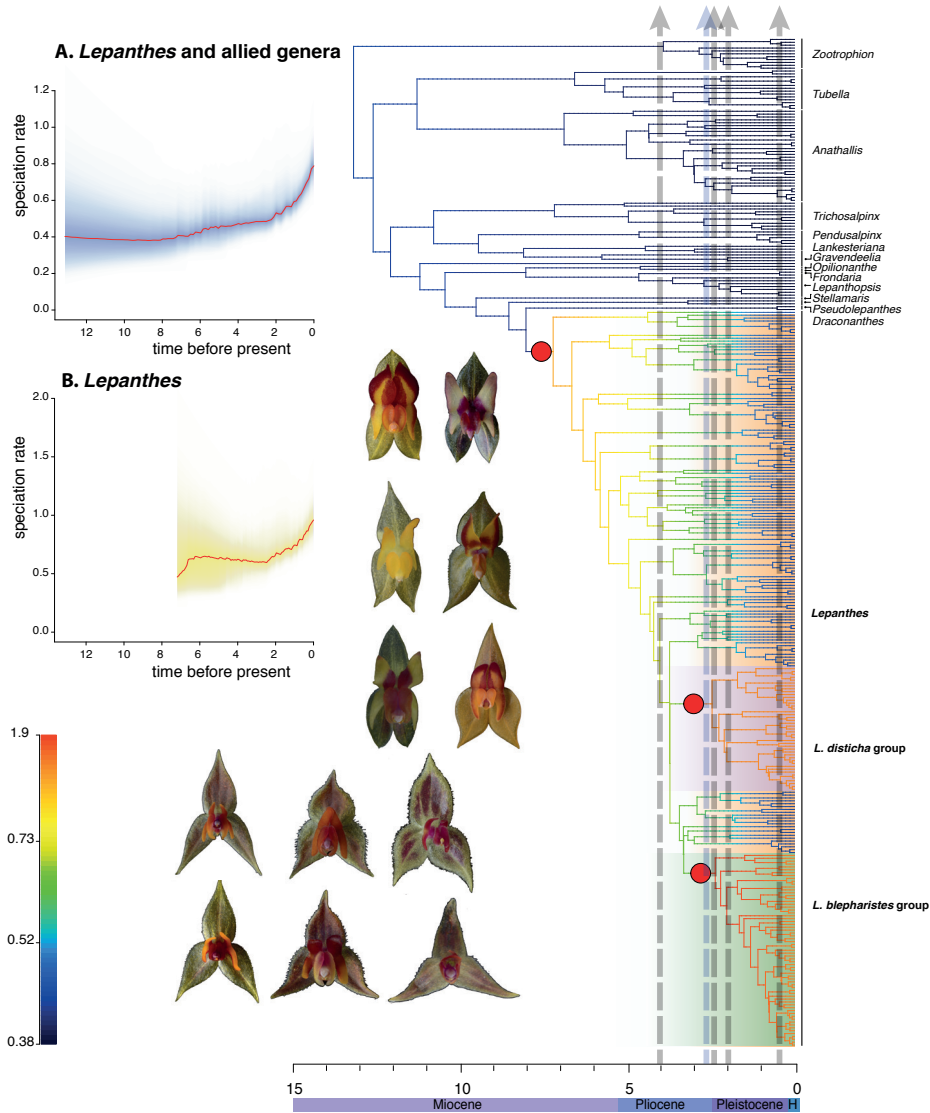
### 9.3.4 Diversification of *Lepanthes* and allies

The BAMM analyses moderately rejected a null model with zero shifts (Bayes factor = 12.75) and identified three rate shifts across the *Lepanthes* clade (Table 9.5). The best model configuration identified three rate shifts, all in the genus *Lepanthes* (Fig. 9.2). The first rate shift, detected in the MRCA of *Lepanthes* around 7 Ma, was also described in Chapter 8. In comparison, the speciation rates of all closely related genera only slightly decreased over time and no shifts in diversification rates were detected, therefore, these groups (e.g. *Anathallis*, *Trichosalpinx*, *Zootrophion*) have not become as diverse as *Lepanthes* (Bogarín et al., 2018c). By increasing the species sampling for *Lepanthes* in the current study, we detected two additional rate shifts corresponding to the *L. disticha* and *L. blepharistes* groups, which occurred almost at the same time at around 2.5 Ma, towards the end of the Pliocene. These two groups are endemic to Costa Rica and Panama and taxonomically complex. Most of the new species of *Lepanthes* described recently belong to these groups (Bogarín et al., 2016; Pupulin and Bogarín, 2012). The high morphological similarity and low sequence variation in ITS and *matK* markers observed suggests a possible correlation with fast and recent within-area speciation as inferred here (Bogarín et al., 2018d).

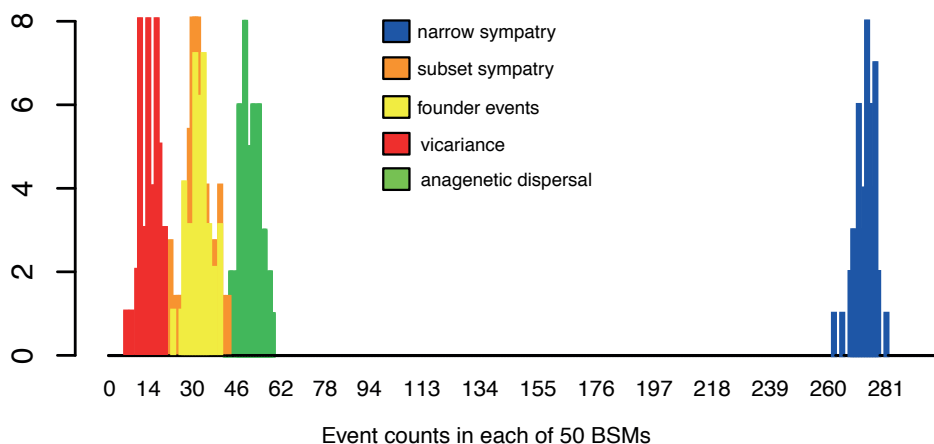
Overall, the rates through time in *Lepanthes* showed an acceleration around 7 Ma and at 2 Ma but these accelerations did not strictly correlate with mountain orogeny (Fig. 9.2). Similar results were observed in the Pleurothallidinae because no correlation was found among palaeo-elevation, mountain uplift and diversifications. The absence of a correlation with orogeny can be

**Table 9.5.** Summary of the biogeographical stochastic mapping with the DEC+J model showing event counts across 50 BSMs, mean of events and standard deviations.

	shifts	Denominator models								
		0	1	2	3	4	5	6	7	8
Numerator of models	0	1.00	0.31	0.08	0.08	0.09	0.14	0.21	0.13	0.19
	1	3.23	1.00	0.27	0.25	0.28	0.44	0.69	0.40	0.61
	2	12.13	3.75	1.00	0.95	1.04	1.65	2.60	1.52	2.27
	3	12.75	3.95	1.05	1.00	1.09	1.74	2.73	1.59	2.39
	4	11.67	3.61	0.96	0.92	1.00	1.59	2.50	1.46	2.19
	5	7.33	2.27	0.60	0.58	0.63	1.00	1.57	0.92	1.38
	6	4.67	1.45	0.38	0.37	0.40	0.64	1.00	0.58	0.88
	7	8.00	2.48	0.66	0.63	0.69	1.09	1.71	1.00	1.50
	8	5.33	1.65	0.44	0.42	0.46	0.73	1.14	0.67	1.00



**Figure 9.2.** Time-calibrated phylogenetic tree of *Lepanthes* and allied genera with the best shift configuration obtained with BAMM analysis and colored according to speciation rate. Three rate shifts were detected, one at the MRCA of the genus, and two at the internal nodes corresponding to *L. disticha* and *L. blepharistes* groups (red circles). Rate-through-time analyses of speciation rates (density shading area indicates 95% Bayesian credible region of the distribution of rates) of (A) *Lepanthes* and allied genera and (A) the genus *Lepanthes* showing an acceleration of speciation rate starting at around 2.5 Ma. Grey arrows show the age of mountain ranges in SCA and the blue arrow the start of a climatic cooling period at about 2.7 Ma (Molnar, 2008). Timescale shown at the bottom is expressed in million years ago (Ma).



**Figure 9.3.** Histograms of the counts of different biogeographical events estimated in each of the 50 BSMs.

the result of the rapid diversification of migrating cool preadapted Pleurothallidinae lineages into already formed montane environments as hypothesized in Chapter 8 (Hoorn et al., 2010; Kirby, 2016, 2007). The mountain ranges of Costa Rica and Panama are among the youngest in the world and emerged as a volcanic island-arc in the Central American Seaway (CAS) around 25 Ma during the Cenozoic. Denyer and Alvarado (2007) calculated the origin of the Costa Rican Guanacaste and Central Volcanic Ranges as less than 0.5 Ma, Fila Costeña as less than 2 Ma and Cordillera de Tilarán as 5 Ma. The Cordillera de Talamanca (containing the highest peaks up to 3820 m.a.s.l.) uplifted to its present elevation during the Pleistocene and Holocene and its age was calculated as around 10-4 Ma. Historically, the closing of the CAS was assumed to start about 12 Ma and to be completed about 4.2 to 3 Ma (Denyer and Alvarado, 2007; Kirby, 2011, 2007). However, relatively new evidence suggests that the CAS closed about 15 to 13 Ma (Hoorn and Flantua, 2015; Montes et al., 2015). In both scenario's, epiphytic orchids were likely able to colonize the CAS volcanic island-arc by their wind-dispersed seeds. Furthermore, paleoclimatic fluctuations are also critical in the establishment of *Lepanthes* species (and likely also the associated pollinators and host trees) because they need humid, cold conditions to survive. Paleoclimatic evidence indicates that climatic cooling periods started before 2.7 Ma in the late Pliocene and early Pleistocene and this partially correlates with *Lepanthes* diversifications in SCA (Molnar, 2008).

Other biological factors responsible for accelerating orchid species diversifications are animal-plant interactions (Givnish et al., 2015). *Lepanthes* employs a sexual deception strategy of pollination but linking pollinator interactions to species diversifications for the entire genus is still impossible because of the scarcity of pollinator observations (only published for three out of >1,200 species) (Blanco and Vieira, 2011; Blanco and Barboza, 2005; Calderón-Sáenz, 2012). In addition, Tremblay and Ackerman (2001) found that genetic drift due to small effective population size and restricted gene flow may play a role in species diversification in *Lepanthes* because the interaction between drift and selection enhance population differentiation. Moreover,

rarity of pollinators or their inability to disperse over long distances might contribute to gene flow restriction among populations (Blanco and Barboza, 2005). Furthermore, other biotic factors such as mycorrhizal associations and availability of endophytic fungi could be limiting factors for seedling establishment in *Lepanthes*. The possible contributions of all these biotic factors to species diversification in *Lepanthes* needs further investigation.

## 9.4 Conclusions

*Lepanthes* likely originated in the Central Andes and diversified between 7-8 Ma in the Miocene. The genus reached Southern Central America from the Andean region twice, with one recolonization to the Northern Andes from Southern Central America. The extant lineages from Northern Central America and the West Indies were likely derived from Southern Central American MRCAs and not from Guyana or Amazonia. The most common biogeographical event was cladogenesis by within-area speciation and the most frequent dispersal routes recorded were Southern Central America with Northern Central America and Northern Andes, and Northern Andes with Central Andes. The genus showed the highest rates of species diversification in the Pleurothallidinae and its most recent Southern Central American clades experienced shifts in species diversification with accelerations around 2.5 Ma. These clades show a high morphological similarity and low variation in the standard DNA markers nrITs and *matK*. Groups derived from recent, rapid diversifications should therefore be analyzed with innovative genomic techniques such as next generation sequencing in order to obtain fully resolved phylogenies (Bogarín et al., 2018d) (Chapter 8). Additionally, their molecular clock age estimates produced only 95% HPD intervals and more accurate estimations are needed. Recently, Sanmartín and Ree (2018) stated that the DEC+J model is a poor model of founder-event speciation, and that statistical comparisons with DEC are inappropriate. Therefore, comparisons with other biogeographical models should be performed to further investigate the consistency of all results obtained. Although our sampling from Southern Central America was the most intensive to date, the sampling of species from Andean, Northern Central America and West Indies lineages is still low and should be further increased. In addition, more accurate species distributions and the discovery of species belonging to new potential lineages will be key to a full understanding of the drivers of speciation and biogeography of *Lepanthes*.

