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Evolutionary diversification in the hyper-diverse montane forests of the tropical Andes: radiation of *Macrocarpaea* (Gentianaceae) and the possible role of range expansion

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The humid mid-elevation montane forests (MMF) of the tropical Andes harbour high levels of plant species diversity, contributing to the exceptional overall diversity of the tropical Andean biodiversity hotspot. However, little is known about the diversification dynamics of MMF plant lineages compared to lineages in other Andean biomes. Here, we use *Macrocarpaea* (Gentianaceae) to investigate patterns of plant diversification in the MMF, using molecular dating with fossils and secondary calibration (from a family-wide phylogenetic analysis of Gentianaceae). We sequenced 76 of 118 recognized *Macrocarpaea* spp. for six markers to reconstruct a time-calibrated phylogenetic tree and infer the historical biogeography of *Macrocarpaea* using maximum-likelihood methods implemented in BioGeoBEARS, estimating diversification rates through time and among lineages with BAMM. We document a rapid radiation of *Macrocarpaea* in Andean MMF coinciding with rapid colonization and range expansion across the entire distribution of the genus in the Andes starting 7.2 Mya. Our results support allopatric founder-event speciation as the dominant process contributing to geographical phylogenetic structure across the genus. We propose that establishment of the MMF in the late Miocene, when the Andes attained critical elevation to modify regional climates, provided large new areas of suitable habitat for *Macrocarpaea* to quickly colonize and expand through repeated founder-events. We suggest that this wave of colonization and range expansion triggered rapid diversification, and, as the MMF became progressively occupied, the diversification rate slowed. Our study also supports the idea that MMF plant radiations are older and more slowly evolving than the quickly evolving lineages in the recent Andean high-elevation grasslands.

ADDITIONAL KEYWORDS: diversification – founder-events – historical biogeography –phylogeny.

INTRODUCTION

Disparities in species diversity between regions of the world and evolutionary lineages have long fascinated evolutionary biologists, as exemplified by the debate surrounding the underlying causes of the latitudinal gradient of species diversity (Jablonski, Roy & Valentine, 2006; Mittelbach *et al.*, 2007). Despite the generality of this broad global-scale pattern, finer regional contrasts are equally striking (Gaston, 2000; Whittaker, Willis & Field, 2001). In this respect, 'centres of angiosperm biodiversity' (Morawetz & Raedig, 2007; Raedig *et al.*, 2010) are of particular interest because their high endemism implies that these regions favour longer species persistence (lower extinction rates) and/or enhanced species origination (higher speciation rates) than elsewhere (Goldberg *et al.*, 2005; Tzedakis, 2009). Disentangling the evolutionary trajectories associated with the assembly of hotspot biodiversity can provide insights into the processes that generate and maintain the highly uneven distribution of species diversity across the planet.

Among terrestrial hotspots, the tropical Andes is the richest in terms of both species diversity and endemism (Orme *et al.*, 2005). This extraordinary diversity of the tropical Andes has been broadly attributed to

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*Corresponding author. E-mail: Jonathan.kissling@unine.ch exceptional overall physiographic (topographic and habitat) heterogeneity, providing a key landscape for hyper-diversification. More specifically, two major historical events prompting species diversification have been highlighted. First, the later phases of Andean uplift during the late Miocene and early Pliocene are thought to have promoted diversification (Hoorn *et al.*, 2010; Antonelli & Sanmartín, 2011a; Luebert & Weigend, 2014; Lagomarsino *et al.*, 2016; Hazzi *et al.*, 2018) through: (1) vicariant fragmentation and isolation of lowland taxa by mountain uplift; (2) allopatric speciation during range expansion of taxa adapted to newly available and greatly expanded montane habitats; and (3) parapatric speciation along the steep and extended ecological gradients that characterize the Andes. Second, Pleistocene climatic oscillations may have further enhanced diversification through allopatric speciation resulting from repeated glacial contractions and interglacial expansions causing a signal of flickering connectivity between populations and species (Hooghiemstra, Wijninga & Cleef, 2006; Rull, 2011; Madriñán, Cortés & Richardson, 2013; Flantua *et al.*, 2014, 2019; Hazzi *et al.*, 2018; Nevado *et al.*, 2018).

It is also clear that the tropical Andean biodiversity hotspot cannot be viewed as a single coherent unit characterized by similar trajectories of evolutionary diversification. The tropical Andes hotspot encompasses diverse environmental conditions (dry to perhumid, warm to freezing, low to high elevation) and sharply contrasting vegetation types and associated biotas. To facilitate macroevolutionary comparisons, three broad biomes were proposed by Särkinen *et al.* (2012), based on overall environmental and floristic similarities. The seasonally dry tropical forests (SDTF; 0–2000 m) encompass the dry-adapted plant communities from the rain-shadowed inter-Andean valleys from Colombia to Bolivia and the western Andean foothills in northern Peru. The humid mid-elevation montane forests (MMF; *c.* 1000–3500 m) comprise the mesic plant communities on the eastern slopes of the Andes from Venezuela to Bolivia and the Pacific slopes of Colombia and northern Ecuador, sandwiched between the lowland tropical rain forests and the tree line (including lower and upper montane forests, subpáramo shrub forests and cloud forests). Finally, the high-elevation grasslands (HEG; *c.* 3000–4800 m) comprise a set of cold-adapted plant communities above the tree line (including páramo, super-páramo, jalca and puna) along the high-elevation Andes from Venezuela to Bolivia (Särkinen *et al.*, 2012). Several studies have addressed the diversification dynamics of plant lineages in the SDTF and the HEG (Pennington *et al.*, 2004, 2009, 2010; Hughes & Eastwood, 2006; Särkinen *et al.*, 2012; Hughes, Pennington & Antonelli, 2013; Madriñán *et al.*, 2013; Hughes & Atchison, 2015). They revealed that the timing, tempo and trajectories of diversification in these two biomes, although both island-like in distribution, differ markedly, suggesting that the two biomes have evolved largely in isolation from one another (Pennington *et al.*, 2010; Särkinen *et al.*, 2012; Luebert & Weigend, 2014; Hughes, 2016). In contrast, and despite the fact that the MMF harbours the greatest diversity in the tropical Andes (Gentry, 1992; Richter *et al.*, 2009; Jørgensen *et al.*, 2011), the evolutionary trajectories of plant species diversification in the MMF biome remain poorly known. This means that the MMF evolutionary trajectory remains less well understood, hindering the formulation of an 'Andean biotic separation hypothesis' for the tropical Andean biodiversity hotspot as a whole (Särkinen *et al.*, 2012).

The MMF is characterized by humid to perhumid forests rich in epiphytes (orchids, bromeliads, ferns, mosses) with a well-developed understorey that receives a significant fraction of its precipitation in the form of water trapped by the vegetation from persistent cloud cover (Hölscher, 2008). Seasonality in precipitation and temperature is low but tends to increase with distance from the Equator. This biome is marked by steep environmental gradients associated with elevation (Beck & Richter, 2008; Gerold, 2008; Kessler *et al.*, 2011) that influence the physiognomy and floristic composition of the vegetation (Leuschner *et al.*, 2013; Asner *et al.*, 2014). In general, plant species richness decreases, whereas endemism increases with increasing elevation (Jørgensen *et al.*, 2011). The combination of steep slopes and high precipitation causes frequent landslides that contribute to the overall physiographic heterogeneity and dynamism of the MMF (Mutke *et al.*, 2014). The MMF biome harbours many species-rich plant groups (Beck & Richter, 2008), including *Epidendrum* L. (Orchidaceae), *Miconia* Ruiz & Pav. (Melastomataceae), *Piper* L. (Piperaceae), *Pilea* Lindl. (Urticaceae), *Solanum* L. (Solanaceae) and *Schefflera* J.R.Forst. & G.Forst. (Araliaceae), suggesting possible radiations. Previous phylogenetic analyses that included Andean MMF taxa (*Fuchsia* L., Berry *et al.*, 2004; *Renealmia* Houtt, Särkinen *et al.*, 2007; *Ceroxylon* Bonpl., Trénel *et al.*, 2007; *Cinchona* L., *Ladenbergia* Klotzsch, Antonelli *et al.*, 2009; Episcieae, Gloxinieae, Perret *et al.*, 2013; *Polystichum* Roth, McHenry & Barrington, 2014) suggested that MMF diversification coincided with the final phase of the Andean uplift during the mid-Miocene to early Pliocene. However, few of these studies had dense enough sampling to investigate geotemporal trajectories of diversification in detail. Several more recent phylogenetic studies have provided more compelling evidence for elevated rates of species diversification associated with Andean

MMF clades, notably *Hedyosmum* Sw. subgenus *Tafalla* (Ruiz & Pav.) Solms (Chloranthaceae) (Antonelli & Sanmartín, 2011b), within the core tillandsioid clade of Bromeliaceae (Givnish *et al.*, 2014), the mostly Andean Neotropical Vaccinieae (Ericaceae) (Schwery *et al.*, 2014), the *Oreinotinus* clade of *Viburnum* L. (Adoxaceae) (Spriggs *et al.*, 2015), Andean *Passiflora* L. supersection *Tacsonia* (Juss.) Feuillet & J.M. MacDougal of (Passifloraceae) (Abrahamczyk, Souto-Vilarós & Renner, 2014), the centropogonid clade of Andean bell flowers (Lagomarsino *et al.*, 2016) and several clades of the two largest Neotropical orchid groups (Cymbidieae and Pleurothallidinae; Pérez-Escobar *et al.*, 2017), suggesting that radiations may be common in the Andean MMF biome.

To further investigate the evolutionary dynamics of plants in the Andean MMF biome we focus on *Macrocarpaea* (Griseb.) Gilg (Gentianaceae: Helieae) to test the following hypotheses: (1) species diversification in the Andean MMF involved rapid radiations, as proposed by Schwery *et al.* (2014) and Lagomarsino *et al.* (2016); (2) MMF plant radiations are older and slower evolving than those in the higher elevation HEG biome, as suggested by Särkinen *et al.* (2012) and Schwery *et al.* (2014); and (3) diversification was driven primarily by opportunities associated with the emergence of MMF habitats and high physiographic heterogeneity linked to the later phases of Andean uplift, or with the impacts of Pleistocene climate oscillations on the distribution of Andean vegetation zones.

Macrocarpaea belongs to tribe Helieae of Gentianaceae (Calió *et al.*, 2017; Struwe *et al.*, 2002, 2009a), and relationships among some of its taxa have already been investigated using preliminary molecular phylogenetic analyses (Struwe *et al.*, 2009b; Vieu, 2015; Afzan *et al.*, 2019). As the most species-rich genus of the tribe, *Macrocarpaea* comprises *c.* 118 species of shrubs and small trees restricted to montane regions of the Neotropics, although this number will probably rise as many new species are still being discovered, especially in the Andes (Grant, 2003, 2004, 2005, 2007, 2008, 2011, 2014a, b; Grant & Struwe, 2001, 2003; Grant & Weaver, 2003; Grant & Trunz, 2011; Grant & Vieu, 2014). Flowers of *Macrocarpaea* are white or yellow to green (Fig. 1) and are visited by many pollinators including bats, moths, hummingbirds and many groups of insects. The woody habit of *Macrocarpaea* is uncommon in the family and probably evolved from suffrutescent perennials (Carlquist & Grant, 2005; Olson *et al.*, 2013).

Eight *Macrocarpaea* spp. occur in the montane Atlantic forests of Brazil, six in the Pantepui of the Guayana Shield, five in Mesoamerica (Costa Rica and Panama) and three in the Greater Antilles. *Macrocarpaea* provides an excellent study group to investigate the evolutionary trajectories of MMF lineages because the majority of species (96, or 81%) occur in the tropical Andes from Venezuela to Bolivia. The genus occupies a broad elevational range in the Andes (500–3500 m) coinciding with the distribution of the MMF biome, but most individual species occupy much narrower elevational ranges. Most species are endemics with narrowly restricted distributions, and even the most widely distributed species do not exceed 250 km in distribution latitudinally. It is common to find several species in sympatry. A previous phylogenetic study that addressed patterns of diversification of *Macrocarpaea* was limited by low phylogenetic resolution and lack of time-calibration (Struwe *et al.*, 2009b). Here, we improve taxon sampling (adding 19 species), enhance phylogenetic resolution using additional molecular markers and reconstruct the first time-calibrated phylogenetic tree for the genus. We use these dated trees to infer the historical biogeography of the genus, compare a set of vicariance and dispersal scenarios regarding range evolution of *Macrocarpaea* in the Andes and estimate diversification rates through time and among lineages.

MATERIAL AND METHODS

Taxon sampling

Seventy-six of the 118 recognized *Macrocarpae*a spp. (64%) were sampled in the field or from herbarium specimens assembled as part of a detailed taxonomic revision of the genus (Struwe *et al.*, 2009b; Grant, 2014a; see Supporting Information, Appendix S1). These species cover the sectional classification of *Macrocarpaea* (Grant, 2005) and the full spectrum of morphological, ecological and geographical variation: 75% (6/8) for the Atlantic forests of Brazil, 17% (1/6) for the Pantepui of the Guayana Shield, 60% (3/5) for Mesoamerica, 100% (3/3) for the Greater Antilles and 65% (62/96) for the Andes. One representative of each species was included alongside five species of *Tachia* Aubl. and one of *Chorisepalum* Gleason & Wodehouse as outgroups based on previous phylogenetic analyses (Struwe *et al.*, 2009a, b).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from silica-dried leaf samples or herbarium specimens with a standard cetyltrimethylammonium bromide (CTAB)–chloroform extraction followed by isopropanol precipitation and ethanol washing following Doyle & Doyle (1990). Two ribosomal DNA intergenic spacers, ITS (the 5.8S subunit and internal transcribed spacers, ITS1 and ITS2) and 5S-NTS (non-transcribed spacer), were sequenced. The ITS region was amplified using

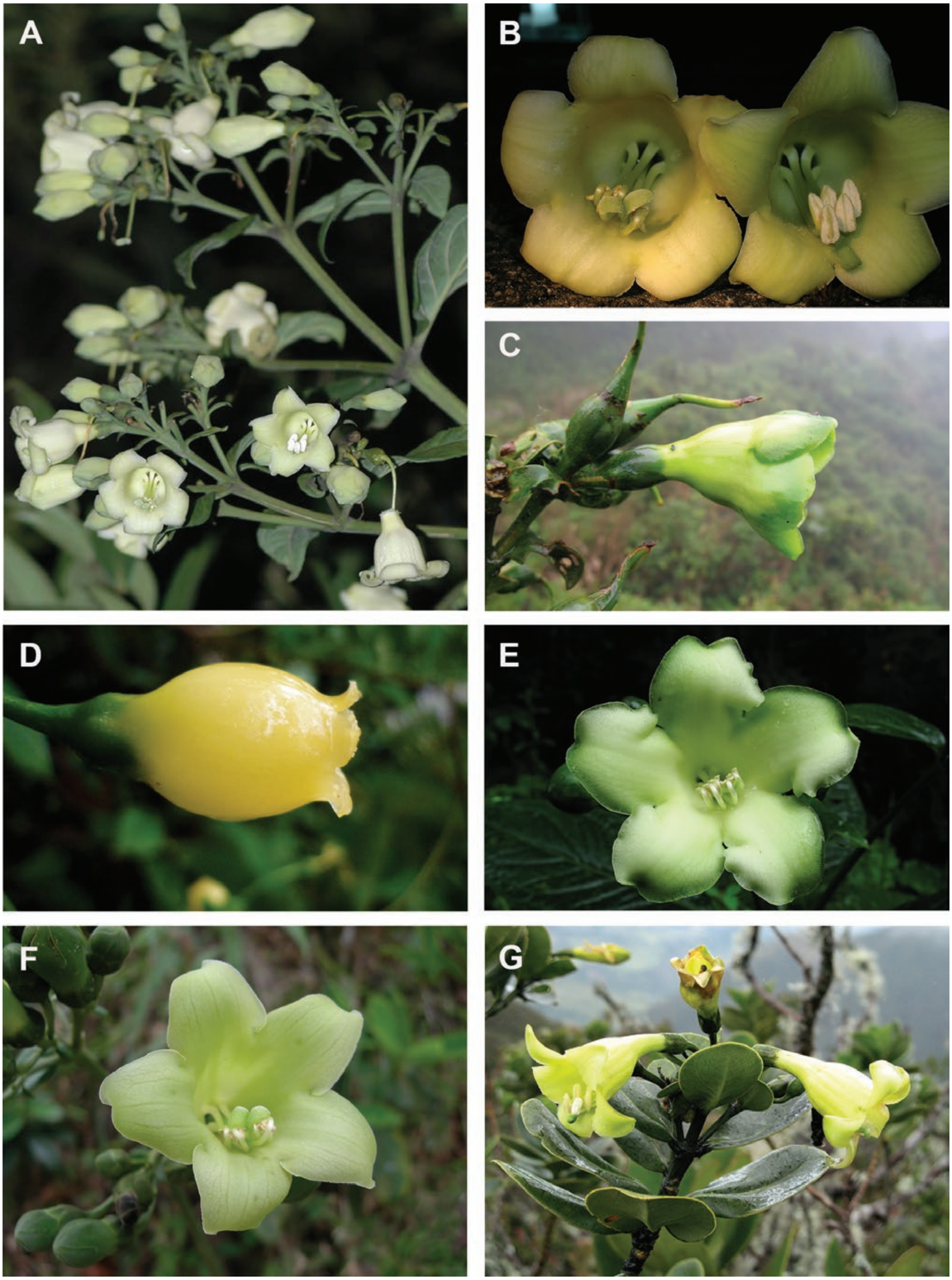

Figure 1. Diversity of flowers in *Macrocarpaea* (Gentianaceae): A, flowering shoot of *M. apparata*; B, *M. apparata* showing two protandrous flowers; C, *M. luna-gentiana*; D, *M. rubra*; E, *M. opulenta*; F, *M. lenae*; G, *M. arborescens*. Pictures by Jason Grant (A, C, E, F, G), Neil Villard (B) and Vincent Trunz (D).

the primers ITS4/ITS5 from White *et al.* (1990); the 5S-NTS was sequenced using the primers PI/PII of Cox, Bennett & Dyer (1992). Four non-coding plastid DNA regions were also sequenced: the *rpl16* intron and the *trnH-psbA* intergenic spacer were amplified using the primers and temperature profiles of Shaw *et al.* (2005); the *trnL-trnF* intron, intergenic spacer and partial exons, and the *rpl32-trnL* intergenic spacer were amplified using the primers and temperature profiles of Taberlet *et al.* (1991) and Shaw *et al.* (2007), respectively.

SEQUENCE ASSEMBLY, ALIGNMENT AND EVOLUTIONARY MODEL CHOICE

The two complementary DNA strands were assembled and edited using Geneious Pro v.5.5.3 (Biomatters Ltd, New Zealand). Alignments were performed with MAFFT (http://mafft.cbrc.jp/alignment/ server/) using FFT-NS-i settings and verified and adjusted manually in MEGA 5.05 (Tamura *et al.*, 2011). Regions of uncertain homology caused by variable indel lengths in 5S-NTS, *trnL-trnF*, *trnH-psbA* and *rpl32-trnL* were removed. The best fitting DNA substitution models were selected for each marker based on the Akaike information criterion (AIC), using jModelTest 2.1.3 (Darriba *et al.*, 2012). As no incongruence among individual gene trees was detected using the test of congruence among distance matrices (CADM, Campbell, Legendre & Lapointe, 2011) or via visual examination of maximum-likelihood (ML) gene trees for each locus (using RaXML v.8.2.12 with a GTRCAT+I model of evolution – Stamatakis, 2014), the alignments of the six genes were concatenated using SequenceMatrix v.1.7.8 (Vaidya, Lohman & Meier, 2011).

PHYLOGENETIC ANALYSES AND DIVERGENCE TIME ESTIMATION

Phylogenetic trees and divergence times were simultaneously estimated using Bayesian Markov chain Monte Carlo (MCMC) searches in BEAST v.1.10.4 (Drummond & Rambaut, 2007) on the online CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010). We assumed a lognormal relaxed clock model (Drummond *et al.*, 2006). Both pure-birth and birth-death models of speciation were evaluated and compared as tree priors with an MCMC-based adaptation of the AIC (AICM, Raftery *et al.*, 2007). The data were partitioned, and both substitution and clock models were unlinked between the different loci. For each locus the substitution model was set to the closest model as selected with jModelTest (Table 1). All other parameters and operators were set to default.

Table 1. Summary data on the sequence matrices used in the divergence time and phylogenetic analyses of Gentianaceae and the '*Macrocarpaea* subclade'

DNA region	Aligned length	V	PPI	%	Model
ITS	794	577	475	99	TN93+G
trnL	653	333	190	61	HKY+G
Total Gentianaceae	1447	910	665		Unlinked
ITS	681	158	85	85	GTR+G
5S-NTS	381	309	256	94	TN93+G+I
rpl16	769	72	35	80	TN93+G+I
trnL	822	88	38	83	GTR+G
trnH-psbA	345	63	37	82	GTR+G
rpl32	896	115	51	55	GTR+G
Total *Macrocarpaea* subclade	3894	805	502		Unlinked

'Aligned length' is the DNA region length retained in the concatenated matrix. V, variable characters; PPI, potentially parsimony-informative characters; %, percentage of taxa sampled for each DNA sequence locus. 'Model' is the best evolutionary model selected with jModelTest.

Because no fossil record exists for *Macrocarpaea*, we used a secondary calibration strategy (Renner, 2005). To do this, we reconstructed a time-calibrated phylogenetic tree for the entire Gentianaceae for which fossil calibrations are available. We downloaded ITS and *trnL* sequences from GenBank representing all major lineages of Gentianaceae (except Voyrieae). Voucher data were extracted from GenBank to allow concatenation of the markers as far as possible at the individual level and not just the species level. No incongruence was detected using the CADM test or via visual inspection of the two gene trees, and therefore both gene alignments were concatenated. The data matrix includes 344 species representing 67 genera of Gentianaceae, 72 *Macrocarpaea* spp. (four species were omitted from these analyses) and seven outgroups from Gentianales with an aligned length of 1447 nucleotides (Supporting Information, Appendix S2, ITS 2% missing, *trnL* 38.7% missing).

Our calibration strategy to estimate divergence times in Gentianaceae differs slightly from previous analyses (Yuan *et al.*, 2005; Favre *et al.*, 2010; Merckx *et al.*, 2013; Pirie *et al.*, 2015; Favre *et al.*, 2016). We deliberately set wide priors on the calibration constraints to take into account uncertainty about fossil ages (Ho & Phillips, 2009). Three calibration points were used to estimate the age of Gentianaceae: (1) a normal prior distribution with a mean of 78 My) and a large standard deviation of 15 My (95% confidence interval of the mean: 53.3–102.7 My) for the root of the tree, reflecting the age estimates for the crown node of Gentianales obtained by Janssens *et al.* (2009)

and subsequently confirmed by more recent studies (e.g. Magallòn *et al.*, 2015; Li *et al.*, 2019); (2) a lognormal prior probability with a minimum bound of 40 My (offset of 40 My) on the crown node of Potalieae, based on fossil pollen similar to *Lisianthius* P.Browne from the mid- to late Eocene of Panama (Graham, 1984); (3) a lognormal prior probability with a minimum bound of 5 My (offset of 5 My) on the *Gentiana* L. crown node, based on a fossil seed from the Pliocene of Thuringia in Germany, showing diagnostic synapomorphies of *Gentiana* (Mai & Walther, 1988). Both lognormal prior probabilities were assigned a mean of 5.0 and standard deviation of 0.5 as true ages are probably older than fossil ages (*c*. 10 My).

In a second analysis, we used posterior age estimates inferred from the time-calibrated phylogenetic tree for Gentianaceae as calibration priors for a more detailed analysis of *Macrocarpaea*. This detailed analysis was conducted using the six-locus dataset and included all 76 *Macrocarpaea* spp. sampled. Because divergence times estimated from the family-level analysis were log-normally distributed, we imposed lognormal prior probabilities to the most recent common ancestor (MRCA) of the '*Macrocarpaea* subclade' *sensu* Struwe *et al.* (2009a, i.e. *Chorisepalum*, *Macrocarpaea* and *Tachia*) (the root of the tree) and to the MRCA of *Macrocarpaea* itself (stem node of *Macrocarpaea*). Offsets, means and standard deviations were set to cover the estimated 95% highest posterior density (HPD) intervals.

Markov chains were run for 50 million generations sampling every 2000 generations and repeated four times under each speciation tree prior for the family- and the genus-level analyses. The AICM was estimated using Tracer v.1.7.1 (Baele *et al.*, 2012) for each replicate independently and did not favour a birth-death model over the Yule model. Stationarity, convergence and effective sample sizes (ESS, > 200) were verified using Tracer v.1.7.1 removing a 10% burn-in, and BEAST log files were combined using LogCombiner v.1.10.4. Mean evolutionary rates and divergence times were calculated using TreeAnnotator v.1.10.4, keeping median heights for nodes.

BIOGEOGRAPHICAL ANALYSES

Geographical areas were defined following Struwe *et al.* (2009b), with the exception of the three cordilleras of the northern Andes that are here grouped together since no clear geographical structure arose from preliminary analyses of these regions, and because we specifically aimed to analyse migration patterns along the natural latitudinal axis of the Andes. Eight areas were delimited (Fig. 3): A, Greater Antilles; B, Mesoamerica; C, the Occidental, Central, Oriental and Mérida Cordilleras of the northern Andes in Venezuela, Colombia and northern Ecuador; D, the Amotape–Huancabamba zone in Ecuador and northern Peru; E, the Cordillera Central in central Peru; F, the Cordillera Central in southern Peru and Bolivia; G, the Pantepui of the Guayana Shield; and H, the montane Atlantic forests of Brazil. The MMF forms a more-or-less continuous belt along the eastern side of the Andes from Venezuela to Bolivia, but recognition of the four Andean areas delimited here is based on known centres of endemism for *Macrocarpaea*.

Parametric historical biogeographical analyses were conducted using BioGeoBEARS (Matzke, 2013, 2014) which implements likelihood optimization of the dispersal–extinction cladogenesis (DEC, Ree *et al.*, 2005) and dispersal–vicariance models (DIVA, Ronquist, 1997). BioGeoBEARS allows users to define models by relaxing assumptions on cladogenetic (sympatry and vicariance) and anagenetic (dispersal, extirpation) range inheritance parameters (Matzke, 2013) and includes a cladogenetic parameter that accounts for founder-event speciation, in which a population establishes itself out of its ancestral range and subsequently differentiates into a new species (Templeton, 2008). The founder-event parameter '*j*' is usually set as an additional free parameter and its *per event weight* is estimated together with the range expansion rate '*d*', the range contraction rate '*e*' and the overall likelihood of the model. The fit of different models to the data can be compared by computation of corrected AIC (AICc) scores and Akaike weights for each model. In this study, we compared four models: DEC, DIVAlike (for likelihood implementation of the DIVA model), DEC+j and DIVAlike+j.

To assess sensitivity to phylogenetic uncertainty, each model was run on the same set of 100 trees, sampled randomly from the posterior distribution of the BEAST analyses, after removing the outgroups. Maximum ancestral range size was set to 4, but we allowed wide distributions (more than two areas) only for exclusively Andean ranges (CDE, CDF, CEF, DEF, CDEF), thus limiting the total number of states to 42 instead of 163. We also set a dispersal matrix penalizing dispersal between non-contiguous areas to 0.5, whereas dispersal between contiguous areas was set to 1. The ML estimates of the relative probability of the ancestral range at the nodes from the 100 trees, obtained under the best biogeographical model, are summarized on the maximum clade credibility (MCC) tree from BEAST, using TreeAnnotator.

To further investigate the ancestral area of the Andean clade, we tested additional models by constraining the likelihood of the ancestral area of the Andean MRCA using the arguments 'fixnode' and 'fixlike' from the function 'define_BioGeoBEARS_run'. We tested seven scenarios in which the Andean MRCA was constrained to be: (1) endemic to the north (C = 1);

(2) endemic to the Amotape–Huancabamba zone (D = 1); (3) endemic to central Peru (E = 1); (4) endemic to southern Peru and Bolivia (F = 1); (5) widespread in the northern half of the range in the Andes (CD = 1); (6) widespread in the southern half of the Andean range (EF = 1); or (7) widespread throughout the Andes (CDEF = 1). We ran these seven scenarios under each of the four previous models on the set of 100 trees. The combination of the best scenario and the best model was evaluated by comparing the distribution of AICc scores for each area combination (N = 28) over the set of trees. The DIVA+j model constantly returned a better fit to the data under each scenario tested. For simplicity we present the results under this model.

Diversification rate analyses

Variation in speciation, extinction and diversification rates through time and among lineages were investigated using BAMM (Bayesian analysis of macroevolutionary mixtures: Rabosky, 2014; Rabosky *et al.*, 2014a), which implements a model of rate variation that assumes that phylogenetic trees are shaped by a discrete number of distinct and potentially dynamic evolutionary speciation and extinction regimes. BAMM tests for a single time-varying diversification process (no events) across the entire phylogenetic tree or two or more distinct time-varying processes (N events) governing evolutionary dynamics across the tree, using reversible jump MCMC to explore this spectrum of alternative models of lineage diversification. The relative probability of each diversification model can be calculated based on its posterior probability and Bayes factors (Rabosky, 2014). BAMM also estimates marginal distributions of speciation and extinction rates for every branch of a phylogenetic tree, thus allowing comparisons of clade-specific diversification trajectories.

We ran BAMM v.2.1 on the MCC tree from the BEAST analysis. We corrected for sampling fractions by assuming that unsampled species belong to the lineage with which they share their geographical location. This assumption is subject to some uncertainty and is imperfect given the non-monophyly of the northern Andes, but has the desirable effect of accounting for the asymmetry in taxon sampling, which is lower for the northern Andes than other areas. Six clades were used to assign unsampled diversity: (1) the genus *Chorisepalum*, with f = 0.2 (one species sampled for five described); (2) the genus *Tachia*, with f = 0.36 (5/14); and (within *Macrocarpaea*) (3) the Brazilian clade, with f = 0.75 (6/8); (4) the Caribbean clade with f = 1 (3/3); (5) the clade including most of the species from the northern Andes, Mesoamerica and the Pantepui of the Guayana Shield, with f = 0.36 (16/42); and (6) the clade including all species from the Amotape–Huancabamba zone, the central Andes and four species from the northern Andes, with f = 0.77 (51/66). We also set f = 0.60 (83/138) for the backbone part of the tree to account for the fact that *Chorisepalum* does not form a clade per se in our phylogenetic analysis (only one species sampled) and is therefore part of the backbone of the tree.

Priors were set using the 'setBAMMpriors' function in BAMMtools (Rabosky *et al.*, 2014b). All other control parameters were set to default values. We ran four independent BAMM analyses with five million generations of MCMC sampling each. Posterior and 'event data' were sampled every 1000 generations. Convergence between runs was assessed by comparing the log likelihood traces from the MCMC outputs and the ESS for the log likelihood and the number of events, after 10% burn-in. Posterior probabilities of diversification models and evolutionary rates through time were extracted and summarized using BAMMtools.

When a slowdown was detected with BAMM, its significance was evaluated by measuring the γ-statistic and applying the Monte Carlo constant rates (MCCR) test in the R package LASER (Rabosky, 2006) using the number of known and unsampled species in the clade, fixing the number of replicates to 10 000.

RESULTS

Phylogenetic relationships

Alignment lengths, numbers of variable and potentially parsimony-informative (PPI) sites are reported for each DNA sequence locus together with the selected evolutionary model and the percentage of sequences available in the complete dataset (Table 1). Overall, the nuclear marker 5S-NTS alone contributes half of the total number of PPI sites. The four plastid markers show useful levels of informative variation when compared with the nuclear marker ITS, with the *trnH-psbA* locus showing the highest ratio of PPI sites to length (11%).

The MCC tree from the BEAST analysis of Gentianaceae (Supporting Information, Appendix S3) shows strong support [posterior probability (PP) > 95] for tribal relationships and is congruent with previous studies (Struwe *et al.*, 2002; Merckx *et al.*, 2013). Helieae are monophyletic (PP = 0.94) with *Prepusa* Mart. as sister to the remaining genera. The MCC tree (Fig. 2) confirms previous results (Struwe *et al.*, 2009b), in which *Macrocarpaea* spp. from Brazil (section *Tabacifolieae* Ewan) form a clade (PP = 1) sister to a large clade (PP = 1) containing the remaining *Macrocarpaea* spp. In this large clade, a subclade composed of most of the species from the northern Andes (Colombia and northern Ecuador)

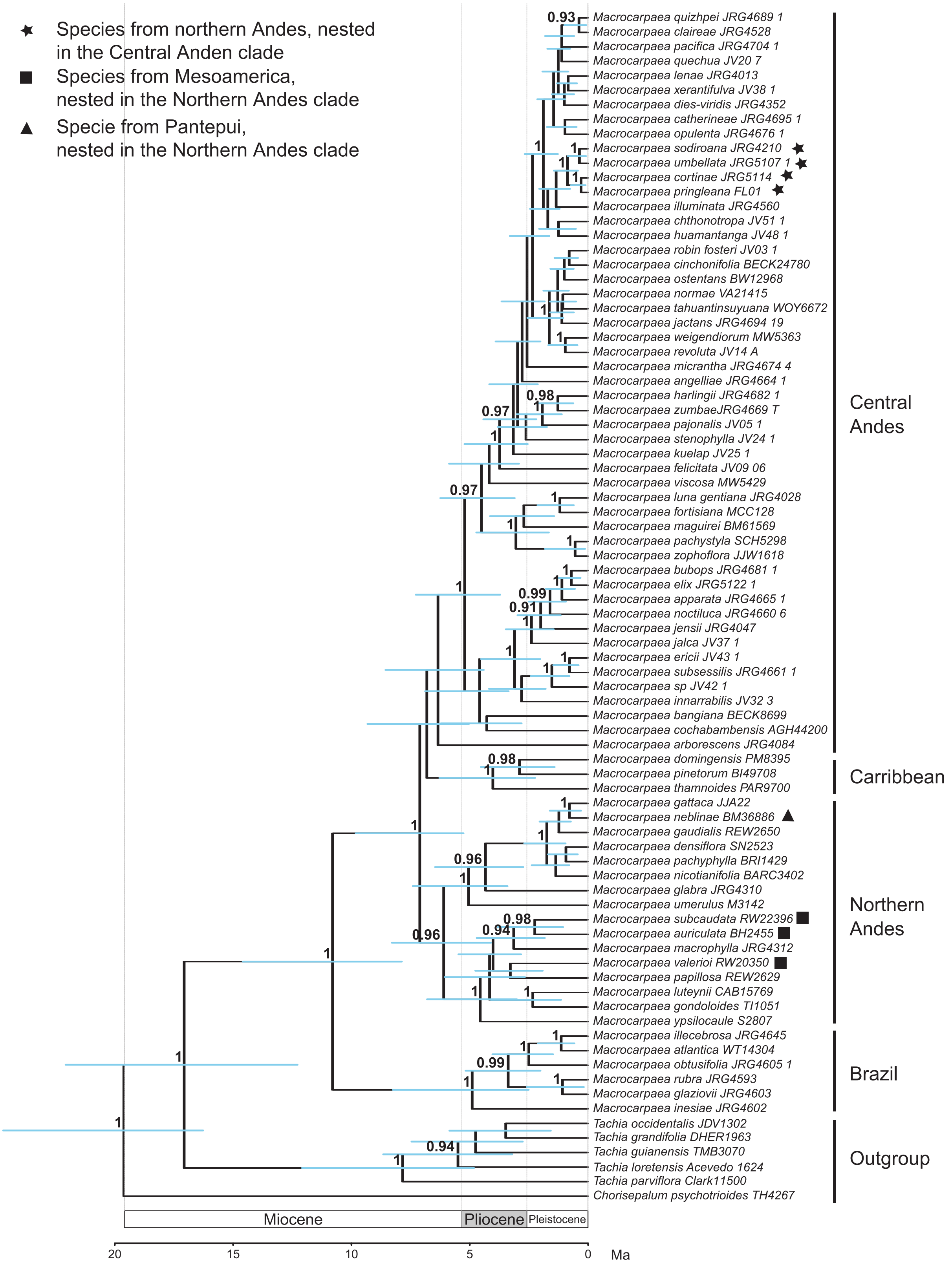

Figure 2. Chronogram of the '*Macrocarpaea* subclade' *sensu* Struwe *et al.* (2009a; i.e. *Chorisepalum*, *Macrocarpaea* and *Tachia*) with 95% highest posterior density bars, based on BEAST analyses of the six-locus dataset (ITS, 5S-NTS,

with species from the Pantepui and Mesoamerica nested in it is robustly supported (PP = 0.96). The Caribbean species form a clade (PP = 1) that is weakly supported (PP = 0.36) as sister to a clade (PP = 1) composed of almost all the species from the Amotape–Huancabamba zone and the central Andes plus a small subclade of northern Andean species nested within it. Relationships among species in the large central/north Andean subclade are generally weakly supported (PP < 0.9) (Fig. 2).

ESTIMATED AGES OF LINEAGES

Age estimates indicate that the '*Macrocarpaea* subclade' diversified in the early Miocene and *Macrocarpaea* itself in the late Miocene and Pliocene (Fig. 2, Table 2). The split between *Prepusa* and the remaining genera of Helieae is estimated to have occurred 36.2 Mya (HPD, 24.7–47.3 Mya), and the MRCA of the Helieae excluding *Prepusa* is estimated at 24.0 Mya (HPD, 17.6–31.1 Mya). In Helieae, the '*Macrocarpaea* subclade' (*Chorisepalum*, *Macrocarpaea* and *Tachia*) started to diversify 20.6 Mya (HPD, 14.0–27.5 Mya), corresponding also to the stem age of *Macrocarpaea*. The estimated crown age of *Macrocarpaea* is 11.1 My (HPD, 7.3–15.1 My). Both stem and crown estimates of the age of *Macrocarpaea* (mean and HPD) were used as priors in the secondary calibration analysis. Overall, these divergence time estimations lie between the younger estimates of Merckx *et al.* (2013) and slightly older estimates of Pirie *et al.* (2015) and provide conservative age estimates for *Macrocarpaea* and its close relatives. The crown age of the Andean clade of *Macrocarpaea* is estimated as 7.2 My (HPD, 5.2–9.8 My).

HISTORICAL BIOGEOGRAPHY

The DIVALIKE+J (with founder-event) model outperformed all other models, returning consistently lower AICc scores (mean AICc = 159.9) and higher Akaike weights (mean ω = 0.749) over the 100 sampled trees (Table 3). The second-best model is the DEC+J model, which according to the criteria of Burnham & Anderson (2002) receives some support (ΔAICc mean = 2.19). The mean evidence ratio (ωbest/ω*i* = 2.992) indicates that the DIVALIKE+J model is almost three times more likely than the DEC+J model. In comparison, models that do not consider founder-event speciation (DEC and DIVALIKE) performed poorly (DEC: ΔAICc mean = 63.607, DIVALIKE: ΔAICc mean = 57.431), suggesting that founder-event speciation is an important process explaining the historical biogeography of *Macrocarpaea*. Under the DIVALIKE+J model, rates of range expansion and range contraction are estimated to be virtually null (mean *d* = 1.92E-12, mean *e* = 6E-11), indicating little or no role for these two anagenetic processes in *Macrocarpaea* range evolution.

Reconstructions of the ancestral area of the MRCA of *Macrocarpaea* remain ambiguous (Fig. 3), with support for combinations of two non-contiguous areas, always including the montane Atlantic forests of Brazil (CH, *P* = 0.39; DH, *P* = 0.32). The ancestral area of the large clade including all Andean species is also ambiguous, with the Amotape–Huancabamba zone (D, *P* = 0.35) and the northern Andes (C, *P* = 0.29) obtaining the most support. Given that anagenetic dispersal was inferred as virtually null in the selected model, dispersal into any new area is associated with the cladogenetic event (founder events) that subtend the nodes for which that particular area is reconstructed for the first time. Results suggest that colonization of the entire Andean range of the genus (areas C, D, E, F) occurred rapidly over *c.* 2 My (7.2–5.2 Mya) primarily from the northern

Table 2. Stem and crown ages (My) for Gentianaceae clades, obtained from BEAST analyses

Clade	Stem age	Crown age
Gentianaceae*	81.99 (66.61, 99.26)	76.84 (61.56, 92.39)
Helieae*	46.63 (37.59, 57.11)	36.17 (24.67, 47.31)
'*Macrocarpaea* subclade'*	23.97 (17.61, 31.07)	20.55 (13.96, 27.48)
*Macrocarpaea**	20.55 (13.96, 27.48)	11.04 (7.29, 15.13)
Tachia†	17.16 (12.23, 22.03)	7.98 (4.77, 12.04)
Brazilian *Macrocarpaea* subclade†	11.03 (7.82, 14.54)	5.11 (2.46, 8.20)
Andean *Macrocarpaea* subclade†	11.03 (7.82, 14.54)	7.16 (5.21, 9.77)

Lower and higher 95% posterior densities are shown in parentheses.
*From the family-level and fossil-constrained analyses based on a two-locus dataset.
†Results from the secondary dating analyses of the '*Macrocarpaea* subclade' based on the six-locus dataset. We note that the Andean *Macrocarpaea* subclade includes species from Mesoamerica, the Caribbean and the Guayana Shield nested within it.

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trnL, trnH-psbA, rpl16 and *rpl32*) and a secondary calibration strategy. See main text for details concerning calibration. Posterior probabilities (PP) are displayed for nodes with PP > 0.9. Nested in the northern Andean clade, three species from Mesoamerica are indicated by a square and one species from the Pantepui by a triangle. Four species from the northern Andes nested in the central Andean clade are indicated by stars.

Table 3. Summary statistics for the biogeographical model selection procedure

Model	*d*	*e*	*J*	AICc	ΔAICc	ω	Evidence ratio	%
DIVALIKE	0.022	4E-03	NA	217.3	57.431	3E-11	3E+14	0
DEC	0.018	0.013	NA	223.5	63.607	1E-13	5E+14	0
DIVALIKE+J	2E-12	6E-11	0.035	159.9	0	0.749	1	100
DEC+J	0	2E-10	0.036	162.1	2.19	0.251	2.992	0

Each model was applied to a sample of 100 posterior trees from BEAST (outgroups were removed), using an ML optimization approach (BioGeoBEARS, R). *d*, anagenetic range-expansion; *e*, range-contraction free parameters; *j*, founder-event cladogenetic free parameter. AICc is the corrected Akaike information criterion for each model. ΔAICc is the AICc difference between the best model and each model. *ω* is the Akaike weight for each model. The evidence ratio is the *ω* of a model divided by the *ω* of the best model. % refers to the percentage of trees for which each model obtained the lower AICc. All values (except %) are means, estimated over the sample of 100 trees. NA, not applicable.

(C, D) to the central Andes (E, F). Colonization of Mesoamerica (B) from the northern Andes (C) occurred *c*. 4 Mya (HPD, 2.8–5.4 Mya), with two subsequent independent dispersal events back to the northern Andes. Despite their proximity and several exchanges between the Amotape–Huancabamba zone (D), the Cordillera Central in central Peru (E), the Cordillera Central in southern Peru and Bolivian Yungas (F), only one recent exchange (1.7 Mya, HPD, 1.15–2.41 Mya) is hypothesized between the Amotape–Huancabamba zone in Ecuador and Peru (D) and the Occidental, Central, Oriental and Mérida Cordilleras of the northern Andes in Venezuela, Colombia and northern Ecuador (C) during the last 6 My.

Across the seven Andean ancestral area scenarios tested, the northern Andes (C) best fits the data (Table 4) for 61 of the sampled trees, followed by the Amotape–Huancabamba zone scenario (D) for 26 trees. Mean AICc differences (ΔAICc mean = −0.008) and Akaike weights for these two scenarios (C, mean ω = 0.441; D, mean ω = 0.409) did not strongly favour one over the other. The mean evidence ratio indicated that the northern Andes scenario is only 1.8 times more probable than the Amotape–Huancabamba zone scenario. Among the other five scenarios, the only one gaining some support was the northern plus Amotape–Huancabamba combined area (CD, ΔAICc mean = 3.578, mean ω = 0.075). This confirmed that even if it is not possible to unequivocally pinpoint a precise region as the ancestral range for the Andean MRCA, it is very likely that it was located in the north (C, D or CD).

DIVERSIFICATION RATES THROUGH TIME

There was strong evidence for significant variation in species diversification rates through time and among lineages across the '*Macrocarpaea* subclade' (Fig. 4A). A two-rate regime model (one rate shift) was favoured by BAMM with a posterior probability of $P = 0.56$ (Fig. 4C). A single rate regime model (no rate shifts) obtained a posterior probability of $P = 0.05$. A posterior odds ratio of 11.3 and a Bayes factor of 23 strongly favour a two-rate over a single-rate regime model (Raftery, 1995). Models with three to five rate regimes have Bayes factor > 10 compared with a single-rate regime model, but a Bayes factor < 0.91 when compared with a two-rate regime model, indicating limited support for further rate heterogeneity across the phylogenetic tree. The location of the shift to higher rates of diversification was most probably located along the branch subtending the Andean clade (marginal odds ratio = 41; Fig. 3D).

The 95% credible set of rate shift configurations, sampled using a Bayes factor criterion of 5 to identify 'true' shifts from shifts expected from the prior alone, contained six shift configurations (cumulative probability $P = 0.95$). By far the most frequently sampled shift configuration ($P = 0.55$) had a single shift at the Andean MRCA (Fig. 3B). The second-most frequently sampled configuration ($P = 0.17$) had a shift at the MRCA of *Macrocarpaea*. The third-most frequently sampled configuration ($P = 0.14$) was a single rate model. Three additional shift configurations were sampled with low frequency ($P = 0.076$, $P = 0.015$, $P = 0.006$). One had a shift along the branch leading to the MRCA of *Macrocarpaea*. The other two included the original shift subtending the Andean clade as well as an additional rate increase (Fig. 4B).

Evolutionary rates-through-time plots confirmed that the marked increase in mean diversification rate *c*. 7.2 Mya was attributable to both an important increase in the speciation rate and a decrease in the extinction rate of the Andean clade (Fig. 5A). Across the remainder of the '*Macrocarpaea* subclade', the mean speciation rate increased from 0.29 species My^{-1} to 0.35 species My^{-1}. The mean extinction decreased from 0.20 species My^{-1} to 0.17 species My^{-1}. This resulted in a mean net diversification rate that increased only slowly through time and remained relatively low across that part of the phylogenetic tree [maximum = 0.19 species My^{-1}, 90% confidence

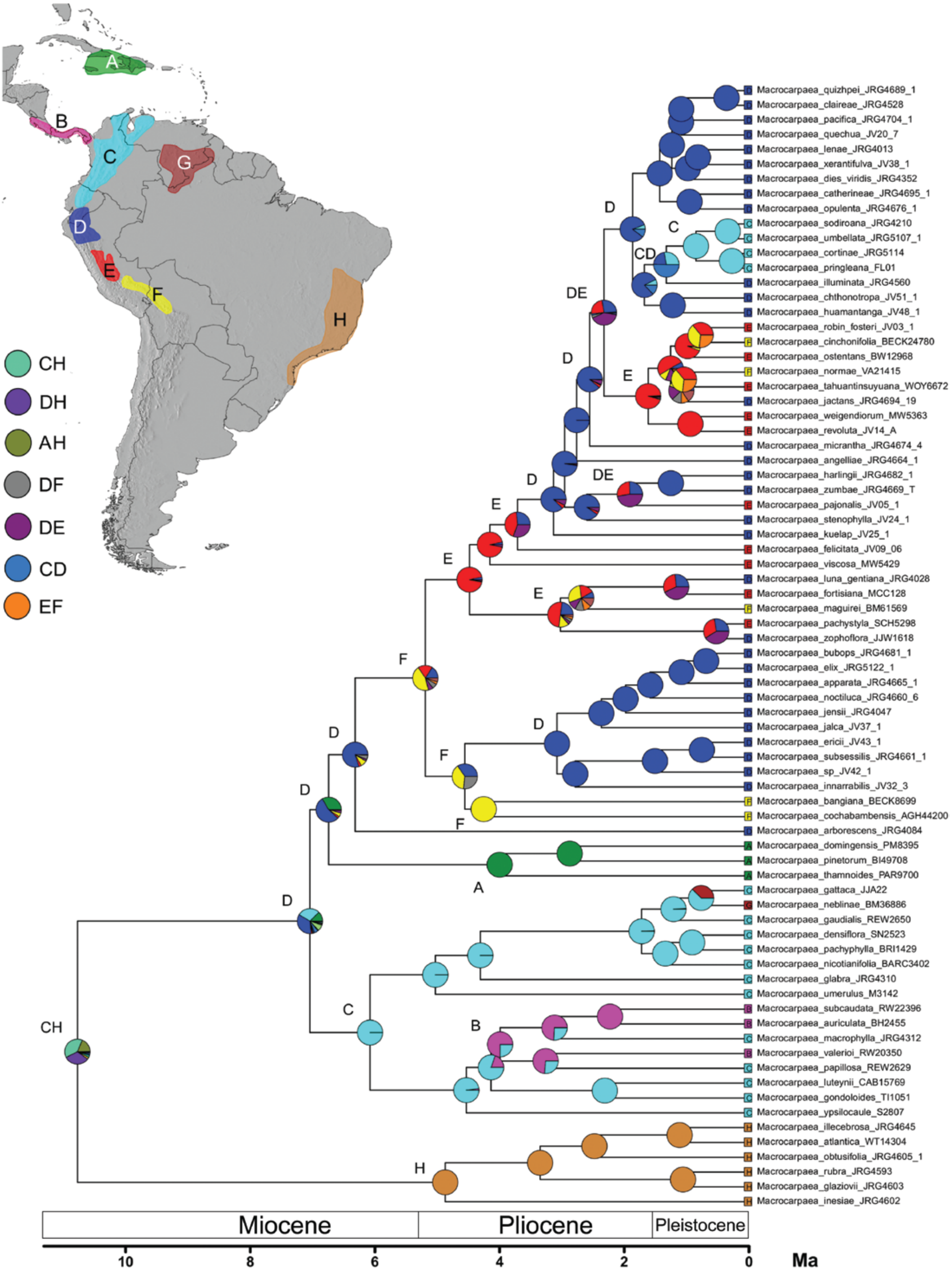

Figure 3. Ancestral area reconstruction obtained using the R package BioGeoBEARS. The DIVALIKE+J model was applied to a sample of 100 dated trees from the BEAST posterior distribution (outgroups pruned), summarized and displayed on the

Table 4. Comparison of scenarios for the ancestral area of the Andean *Macrocarpaea* clade

Scenario	AICc	ΔAICc	ω	Evidence ratio	%
C	162.7	0	0.441	1	61 (9)
D	162.7	−0.008	0.409	1.792	26 (10)
E	170.9	7.610	0.012	133.796	0
F	169.6	6.832	0.047	209.383	3 (1)
CD	166.3	3.578	0.075	8.943	0
EF	176.5	13.788	10E-4	5554.218	0
CDEF	171.3	8.604	0.016	779.071	0

Scenarios involved constraining the state of the MRCA as one of seven possible areas (on 100 trees, with BioGeoBEARS, R). C, D, E, F and their combinations refer to the areas depicted in Figure 4. For an explanation of AICc, ΔAICc, *ω*, Evidence ratio and % refer to Table 3. In parentheses is the percentage of trees for which two scenarios obtained identical AICc.

interval (CI): 0.03–0.36 species My^{-1}] (Fig. 5C). In contrast, the Andean clade had a speciation rate that started at 0.64 species My^{-1} (90% CI: 0.39–0.95 species My^{-1}) and reached 0.50 species My^{-1} at the present (90% CI: 0.40–0.59 species My^{-1}). The extinction rate for this clade started at 0.07 species My^{-1} (90% CI: 0.01–0.17 species My^{-1}) and increased slightly to reach 0.08 species My^{-1} at the present (90% CI: 0.01–0.18 species My^{-1}). As a consequence, the mean diversification rate of the Andean clade started with a relatively high rate of 0.57 species My^{-1} (90% CI: 0.33–0.86 species My^{-1}) and decreased through time to the present-day rate of 0.42 species My^{-1} (90% CI: 0.32–0.52 species My^{-1}) (Fig. 5B). Thus, variation in net diversification rate through time was essentially driven by variation in the speciation rate.

The means of the posterior distribution of diversification rates for *Tachia*, the Brazilian clade and the Andean clade were 0.16 (speciation = 0.33, extinction = 0.17), 0.23 (speciation = 0.37, extinction = 0.14) and 0.44 (speciation = 0.52, extinction = 0.08) species My^{-1} respectively. Nevertheless, the estimated mean diversification rate for the Andean clade is somewhat misleading because diversification rates vary in this clade. The γ-statistic and the MCCR test confirmed the significance of the rate slowdown detected within the Andean clade (known species = 111, unsampled species = 41) by BAMM (MCCR-test, γ-statistics = −3.02, critical value of γ = −2.64, *P* = 0.02).

DISCUSSION

We present evidence for a rapid late Miocene to Pliocene radiation in the Andean MMF biome based on a densely sampled phylogenetic tree for a species-rich MMF plant lineage. Our results show that the radiation of *Macrocarpaea* is associated with rapid colonization and range expansion across the entire extant distribution of the genus in the Andes starting *c.* 7.2 Mya (HPD: 9.7–5.2 Mya). Furthermore, our results suggest that Pleistocene climatic oscillations did not affect the diversification rate of *Macrocarpaea* across the Andes as a whole, but potentially further enhanced diversification in a derived subclade of predominantly lower elevation (< 1800 m) Andean species.

HISTORICAL BIOGEOGRAPHY OF *MACROCARPAEA*

Our results suggest a disjunct distribution including the montane Atlantic forests of Brazil, the northern Andes or the Amotape–Huancabamba zone for the ancestral area of the genus. Connections between the montane Atlantic forests of Brazil and the Andes are common for many Andean-centred plant taxa *sensu* Gentry (1982). Several well-documented examples (Berry *et al.*, 2004; Perret, Chautems & Spichiger, 2006; Givnish *et al.*, 2011; Thode, Sanmartín & Lohmann, 2019) have indicated possible exchange through potential corridors of favourable habitat between Brazil and the Bolivian Andes in the past. Here we provide another example of these connections between the Atlantic forests of Brazil and the Andes, but the direction and the nature of this transition cannot be determined.

Based on the most probable ancestral state for the Andean clade and colonization sequence, we hypothesize a predominantly southward wave of colonization, range expansion and 'dispersification' (*sensu* Moore & Donoghue, 2007) of *Macrocarpaea* along the tropical Andes. Strikingly, our results suggest that the entire extant distribution of the genus in the Andes was rapidly colonized within just *c.* 2 My (5.2–7.2 Mya). Although many details of Andean uplift history remain controversial (Garzione *et al.*, 2008;

Macrocarpaea MCC tree. Pie charts represent the average relative probabilities of the ancestral state at each node. Areas: A, Greater Antilles; Caribbean, green; B, Mesoamerica, pink; C, Cordilleras of the northern Andes in Venezuela, Colombia and northern Ecuador, cyan; D, Amotape–Huancabamba zone in Ecuador and Peru, blue; E, Cordillera Central in central Peru, red; F, Cordillera Central in southern Peru and Bolivia, yellow; G, Guayana Shield, dark brown; H, montane Atlantic forests of Brazil, light brown. Ranges that encompass two or more areas have colours that are a mix of the colours of the areas that compose them (see key next to the map).

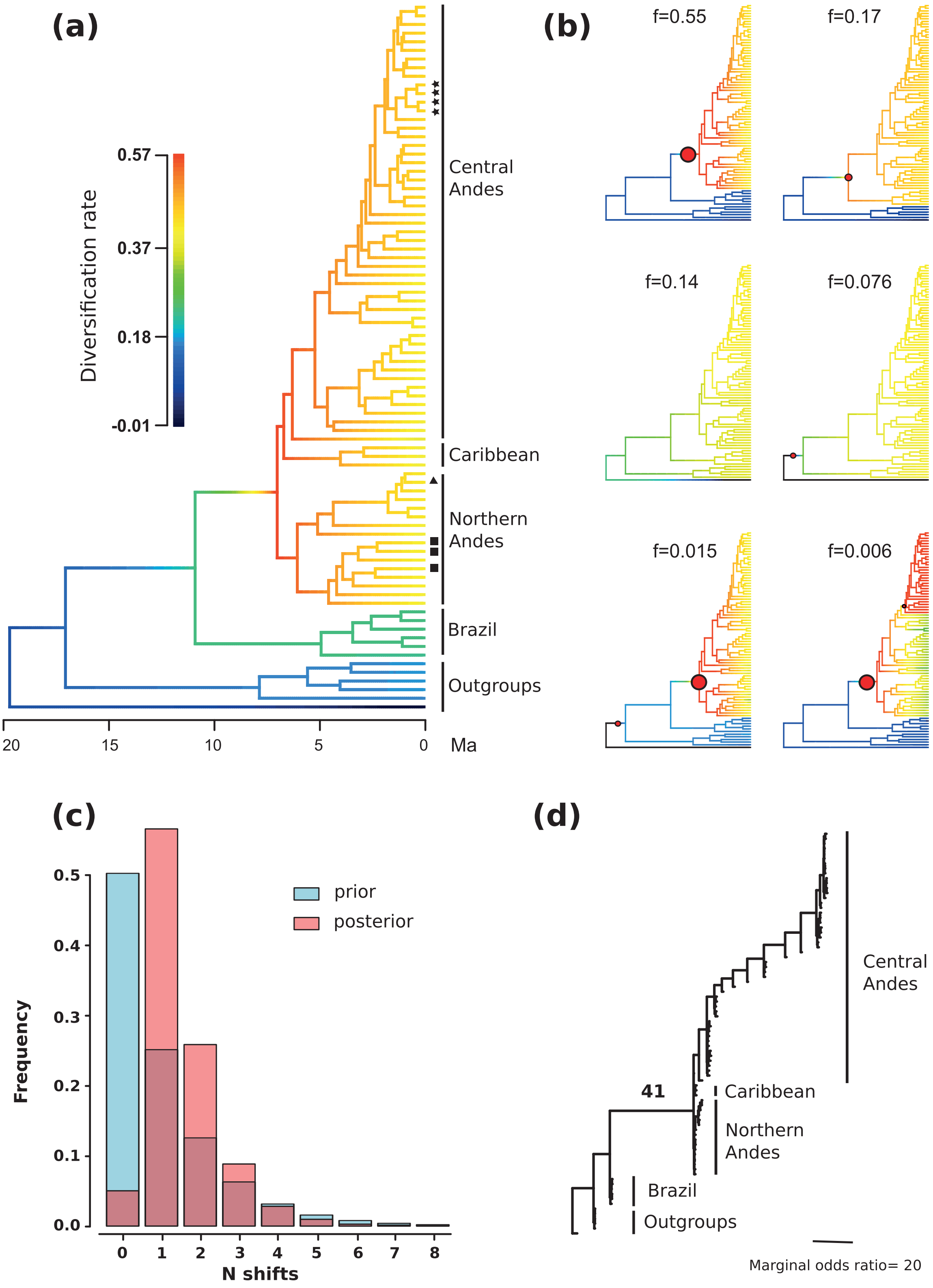

Figure 4. Species diversification rates through time and among lineages of *Macrocarpaea*. Results from Bayesian analysis of macroevolutionary mixtures (BAMM) applied to the MCC time-calibrated tree of the '*Macrocarpaea* subclade'. A, Mean

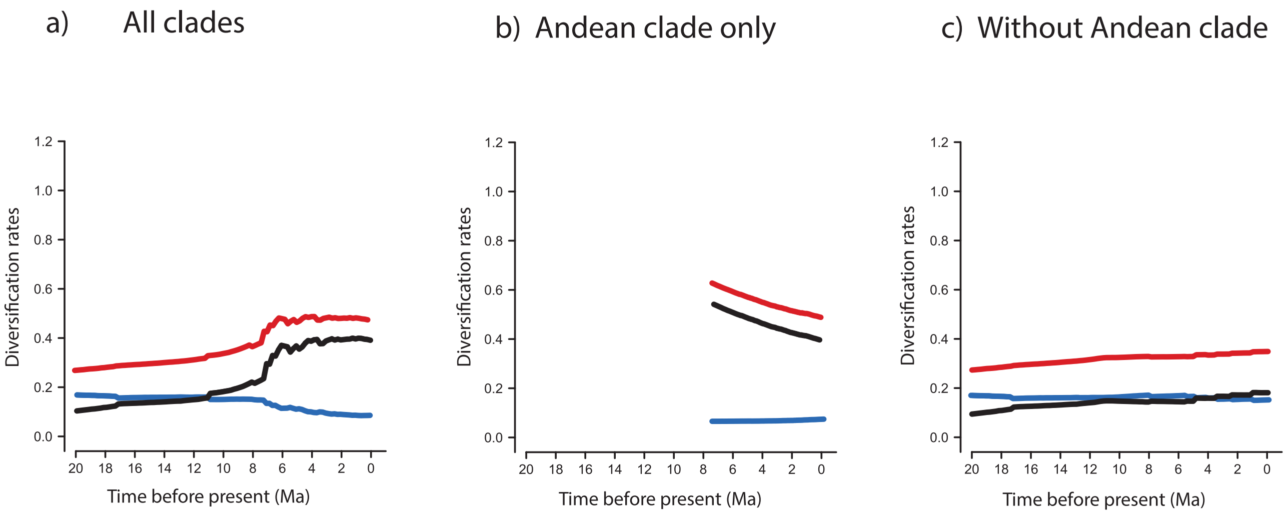

Figure 5. Variation in diversification rates through time: A, across '*Macrocarpaea* subclade' as a whole; B, for the Andean *Macrocarpaea* clade; C, excluding the Andean clade. Black lines represent the mean and the grey polygons the 0.1–0.9 quantiles of the posterior distributions of the diversification rates through time.

Poulsen, Ehlers & Insel, 2010; Luebert & Weigend, 2014, Hazzi *et al.*, 2018), it is widely recognized that the northern South American climate was profoundly altered by the uplift of the Andes in the late Miocene (Hoorn *et al.*, 2010). It is thought that the onset of convective precipitation and the massive increase in rainfall along the eastern flanks of the Andes occurred once the Andes reached *c.* 50–70% of their modern elevation, promoted by transport of Amazonian moisture toward the Andes (Insel *et al.*, 2012). This regional climate change is likely to have triggered and enabled the establishment of widespread humid mid-elevation montane forests in the tropical Andes (van der Hammen & Cleef, 1986). Late Miocene palynological records from the northern Andes (Hooghiemstra *et al.*, 2006) and from the eastern cordillera in northern Bolivia (Graham *et al.*, 2001) together with the divergence time estimates for several plant groups currently inhabiting the MMF (*Ceroxylon*, Trénel *et al.*, 2007; *Cinchona* and *Ladenbergia*, Antonelli *et al.*, 2009; Episceae, Perret *et al.*, 2013; the *Tacsonia* clade of *Passiflora*, Abrahamczyk *et al.*, 2014; core tillandsioids of Bromeliaceae, Givnish *et al.*, 2014; the *Oreinotinus* clade of *Viburnum*, Spriggs *et al.*, 2015; the centropogonid clade of Andean bell flowers, Campanulaceae, Lagomarsino *et al.*, 2016) support this idea (Table 5). The age we infer for the wave of colonization and range expansion associated with dispersal of *Macrocarpaea* in the Andes corresponds to this period, suggesting that *Macrocarpaea* may have invaded the whole range of the Andean MMF soon after it became established. *Macrocarpaea* spp. produce numerous dust-like seeds (0.2–1.5 × 0.2–2.0 mm; Grant, 2005) that are readily transported by wind across considerable distances. This high dispersal ability might have contributed to the rapid southward colonization of the Andean MMF by *Macrocarpaea*.

The multiple transitions inferred between the Amotape–Huancabamba zone and regions further south support the conclusions of Weigend *et al.* (2002), Luebert & Weigend (2014) and Quintana *et al.* (2017) who found that the Amotape–Huancabamba zone did not present a major dispersal barrier for mid-elevation taxa. Instead, we identify the Giron–Paute trans-Andean valley, which coincides with the northern boundary of the Amotape–Huancabamba zone (Keating, 2008), as a potentially important dispersal barrier for *Macrocarpaea*. If we exclude the transition

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phylorate plot showing model-averaged diversification rates at any point along every branch. Nested in the northern Andean clade, three species from Mesoamerica are indicated by a square and one species from the Pantepui by a triangle. Four species from the northern Andean nested in the central Andes clade are indicated by stars. B, The 95% credible set of shift configurations obtained using a Bayes factor criterion of 30; 'f' indicates the posterior probability for each shift configuration. Locations of shifts to higher rates of diversification are indicated by a circle with the size proportional to the marginal probability of the shift. C, Posterior probabilities of the number of regimes in the model sampled. D, Bayes factor support for a rate shift on each branch across the tree, with branch lengths scaled according to the Bayes factor support for a rate shift along the branch. The branch with the likeliest rates of diversification shift is the branch subtending the Andean clade (marginal odds ratio = 41).

Table 5. Comparison of size, age and diversification rates for Andean MMF plant radiations for which there are well-sampled time-calibrated phylogenies

Clade	Number of species	Crown age (My)	Diversification reported (species My^{-1})	Diversification rate estimated (species My^{-1})	Reference
Andean *Macrocarpaea*	96	7.2 (5.2–9.8)	0.43	0.54 (0.74–0.40)	This paper
Andean bell flowers – the centropogonid clade (Campanulaceae: Lobelioideae)	550	5.0 (3.9–6.1)	0.65–1.42	0.82–1.15	Lagomarsino *et al.* (2016)
Andean *Oreinotinus* clade of *Viburnum*	16	6? (NA–NA)	NA	0.35 (NA–NA)	Spriggs *et al.* (2015)
Core tillandsioid clade	1236	9.6 (NA–NA)	0.67	0.67 (NA–NA)	Givnish *et al.* (2014)
Supersection *Tacsonia* of *Passiflora*	62-64	8.4 (6.2–11.2)	NA	0.41 (0.56–0.31)	Abrahamczyk *et al.* (2014)
Hedyosmum subgenus *Tafalla*	40	15 (10.8–29.1)	0.13	0.20 (0.28–0.10)	Antonelli & Sanmartín (2011b)

Diversification rates reported are estimates reported in the original study. Diversification rates were estimated by using Magallón & Sanderson (2001) equation 4 [$r = \log(n) - \log(2)/T$, where n = standing diversity, and T = inferred crown clade age] in the R package GEIGER. Confidence intervals when available are displayed in parentheses. NA, not applicable.

that happened early in the history of the Andean clade (7.2 Mya), only one subsequent exchange is inferred, and rather recently, 1.4 Mya (HPD, 0.71–2.02 Mya), between the Amotape–Huancabamba zone and the northern Andes. Another feature of *Macrocarpaea* biogeography is the colonization of Mesoamerica from the northern Andes 4.3 Mya (HPD, 2.62–6.0 Mya), which, although potentially in line with the ideas of earlier closure of the Isthmus of Panama (Farris *et al.*, 2011; Montes *et al.*, 2015), would probably still have involved long-distance dispersal from the Andes to the nearest montane forests in Mesoamerica.

PROCESSES INVOLVED IN *MACROCARPAEA* RANGE EVOLUTION

The biogeographical analyses strongly favour models integrating a parameter for founder-event speciation, which is known to be an important process in island-like habitats where dispersal to a new area is immediately accompanied by a strong reduction of gene flow potentially because of the lack of habitat connections (Paulay & Meyer, 2002; Cowie & Holland, 2006). This is somewhat surprising because even though it is well established that the SDTF and HEG biomes adjacent to the MMF in the tropical Andes occupy island-like distributions (Hughes & Eastwood, 2006; Särkinen *et al.*, 2012), the MMF has a generally continuous distribution along the eastern flank of the cordilleras. The apparently important contribution of founder-events to *Macrocarpaea* range evolution and the extremely low rates of anagenetic (along the branches) range expansion we infer suggest that the different areas of the Andean MMF (as defined here) could potentially have been less continuous in the past, but there is no evidence for this. It is more likely that connections were constricted along the narrow ‘contour-like’ distribution of the MMF along the eastern flanks of the Andes and that this could have limited dispersal and promoted isolation by distance within a continuous but narrow distribution (see below).

DIVERSIFICATION PATTERNS IN *MACROCARPAEA*

We found evidence for diversification rate heterogeneity through time and among lineages across the phylogenetic tree for *Macrocarpaea*. The root of the phylogenetic tree, the outgroups and the Brazilian clade follow a trajectory of slow and only slowly increasing diversification rates (< 0.13 species My^{-1}). A significant shift to higher rates of diversification is consistently detected along the branch leading to the ancestor of the Andean *Macrocarpaea* clade. This diversification rate regime starts with a relatively high diversification rate of 0.81species My^{-1}, a > 6× increase over the background diversification rate. Subsequently, the diversification rate decreases almost exponentially to the current rate of 0.34 species My^{-1} (Fig. 5B). This pattern of an explosive early burst of high diversification followed by a rate slowdown is typical of a diversity-dependent model of diversification often considered to be the hallmark of evolutionary radiations (Rabosky & Lovette, 2008; Givnish *et al.*, 2011). According to the ecological theory of radiations, the initial burst of diversification is driven by rapid diversification across newly available ecological niches and the slowdown results from the progressive filling of that space as species accumulate (Glor, 2010; Losos, 2010). This rapid early burst of diversification may be accompanied by adaptive ecological 'disparification' and hence adaptive radiation (Gavrilets & Losos, 2009), or may be predominantly driven by non-adaptive geographical processes, such as rapid range expansion across multiple barriers and nearly simultaneous formation of many isolated populations (Moore & Donoghue, 2007, Rundell & Price, 2009; Moen & Morlon, 2014). As the availability of suitable habitats is reduced, geographical opportunities for isolation will decrease and can lead to a decrease in the diversification rate (Pigot *et al.*, 2010).

The diversification rate shift we infer for the Andean clade coincides with the start of the rapid colonization and range expansion of *Macrocarpaea* throughout its current range in the Andean MMF some 7.2 Mya. The apparently rapid appearance and establishment of the MMF along the tropical Andes during the late Miocene and Pliocene (van der Hammen & Cleef, 1986) could have provided the opportunity for *Macrocarpaea* to extend its range. Furthermore, it is postulated that Andean palaeoelevations were at least 50% of the modern elevations with the complex physiography that characterizes this region already in place at that time (Insel *et al.*, 2012). Thus, range expansion of *Macrocarpaea* along the Andes almost certainly occurred across an area of high physiographic heterogeneity that could have prompted isolation of newly formed populations and promoted rapid species diversification, as suggested for montane plant radiations more generally (Schwery *et al.*, 2014). This idea is consistent with the support we found for founder-event speciation as an important process involved in range evolution of *Macrocarpaea*, but the nature of the link between this long-distance dispersal parameter and founder-event as a speciation process remains to be assessed in more detail. The Andean MMF occupies a predominantly narrow linear distribution along a latitudinal axis and can be regarded as a stepping-stone metapopulation system (Trénel *et al.*, 2008). Simulation studies have shown that range expansion through repeated founder-events in the presence of low inter-population gene flow in this type of metapopulation system tends to favour rapid genetic differentiation of populations along the expansion front (Le Corre & Kremer, 1998; Pigot *et al.*, 2010). Thus, the narrow linear distribution of the MMF is likely to have contributed to the accelerated rate of diversification rate during range expansion of *Macrocarpaea*. As *Macrocarpaea* progressively expanded throughout the Andean MMF, opportunities for isolation via the colonization of unoccupied areas by the genus would have decreased, in turn slowing the rate of species diversification.

We have emphasized how the geographical processes that probably operated during range expansion in the Andes could have regulated the evolutionary dynamics of *Macrocarpaea*. However, the Andean MMF are also characterized by steep environmental gradients associated with elevation (Kessler *et al.*, 2011). Ecological gradients promote adaptive divergence (Doebeli & Dieckmann, 2003), and it seems probable that adaptation to different ecological conditions in conjunction with geographical processes has contributed to the rapid diversification of *Macrocarpaea* in the Andes, but rigorously testing this is beyond the scope of the present study. The only clues to evaluate the relative contributions of ecological and geographical processes to the Andean clade diversification come from species pair comparisons. Most putative sister species share similar habitats and are endemic to the same region, but are locally allopatric, favouring geography over ecology as the primary driver of species divergence. Nevertheless, it is also possible that ecological 'disparification' could have occurred in the initial phase of adaptive radiation (Gavrilets & Losos, 2009), such that sister species comparisons which only capture the later phases of radiation might be misleading (Ackerly, Schwilk & Webb, 2006). Additional studies to investigate niche evolution of *Macrocarpaea* in the Andes are needed to evaluate the adaptive component of this radiation.

The impacts of Pleistocene climatic oscillations on species distributions, by promoting repeated allopatric/parapatric speciation, have often been considered as a potential driver of diversification, especially in the high-elevation HEG of the Andes (Rull, 2011; Turchetto-Zolet *et al.*, 2013; Flantua *et al.*, 2019). We did not detect any diversification pulse across the Andean clade as a whole during the last 2.6 My. Nevertheless, the two less frequently sampled rate shift configurations from the BAMM analyses suggest the possibility of a second nested shift to even higher rates of diversification in a derived subclade of mainly lower elevation sub-Andean forest species (Fig. 4B). The age estimates of these additional shifts are 3.1 and 2.3 Mya, respectively, i.e. close to the start of the Pleistocene. This suggests that Pleistocene climatic oscillations might have further enhanced the diversification of Andean species at lower elevations, but not in the higher elevation vegetation belts. Palaeobotanical studies have revealed that among the MMF vegetation belts, it was the lower montane (sub-Andean) forests which experienced the greatest contraction at the Last Glacial Maximum (LGM, Hooghiemstra & Van der Hammen, 2004; Flantua *et al.*, 2014, 2019). Cycles of strong contraction during glacial and re-expansion during interglacial periods throughout the Pleistocene could have promoted episodes of ecological speciation across the upper and lower MMF zones.

These findings for *Macrocarpaea* are remarkably congruent with results for other Andean MMF clades, and there is growing evidence that late Miocene/Pliocene radiation played a central role in generating the present hyperdiversity of the Andean MMF biome. A suite of other MMF plant radiations strikingly coincident in timing and geography, and with similar accelerated rates of species diversification, have been documented (Table 5).

Finally, the age we infer for the onset of radiation in *Macrocarpaea* in the Andes is intermediate between the ages of divergence reported for the older SDTF and younger HEG plant lineages (Särkinen *et al.*, 2012; Hughes, 2016). The diversification rate is also intermediate between those of these adjacent biomes (Särkinen *et al.*, 2012; Madriñán *et al.*, 2013; Hughes, 2016). The estimated diversification rate for *Macrocarpaea* at its peak (0.81species $\text{My}^{-1,}$ 90% CI: 0.31–1.39 species My^{-1}) is *c*. 3× lower than the diversification rate of the HEG genus *Lupinus* that was estimated using a time-constant approach (Drummond *et al.*, 2012), which potentially minimizes the contrast. The high diversification rate inferred for the HEG has been explained by the fact that plant lineages in this young biome might still be in the early explosive phases of radiation (Madriñán *et al.*, 2013; Hughes & Atchison, 2015). This is exemplified by the large Andean HEG radiation of *Lupinus*, in which diversification rates are still accelerating towards the present, suggesting a radiation in the early explosive phase (Hughes & Atchison, 2015). In contrast, *Macrocarpaea* presents a classical trajectory of evolutionary radiation with a rapid initial burst of diversification followed by a rate slow down (Fig. 5). These results suggest that both the HEG and MMF biomes harbour significant plant radiations showing elevated rates of species diversification, but that the lower elevation MMF radiations are older, slower evolving and more mature that those in the HEG. In sharp contrast, the Andean SDTF has been characterized as a 'museum' of diversity in which older lineages have slowly accumulated limited species diversity from the mid-Miocene onwards (Pennington *et al.*, 2010; Särkinen *et al.*, 2012; Hughes, 2016).

CONCLUSIONS

In this study we show that *Macrocarpaea* diversified in the Andean MMF following a classical early-burst evolutionary radiation model, starting with a high rate of diversification followed by an exponential slowdown through time. The radiation of *Macrocarpaea* probably coincided with the initial establishment of MMF along the entire eastern flank of the tropical Andes once palaeoelevations were sufficient to cause high year-round precipitation. This establishment and expansion of the MMF biome is likely to have provided an opportunity for pre-adapted *Macrocarpaea* palaeospecies to disperse, expand and diversify. We propose that geographical processes arising from habitat constriction and fragmentation, and repeated rare dispersal events (founder-events), may have played important roles in driving the radiation. Nevertheless, a potential concomitant role of ecological processes remains to be assessed. Our study also suggests that the MMF biome is a 'mature cradle' of diversity that harbours plant lineages in the later phases of their radiations, thereby confirming that the three prominent biomes of the tropical Andes have contrasting evolutionary histories. This calls for careful consideration of geographical biodiversity units when trying to explain patterns of biodiversity distributions in this prominent global biodiversity hotspot.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. List of taxa sampled and analysed in the *Macrocarpaea* subclade phylogenetic analysis and their corresponding GenBank accession numbers.
Appendix S2. List of taxa used in the Gentianaceae phylogenetic analysis and their corresponding GenBank accession numbers.
Appendix S3. Time-calibrated phylogenetic tree for Gentianaceae.