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Temporal and spatial origin of Gesneriaceae in the New World inferred from plastid DNA sequences

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Gesneriaceae are represented in the New World (NW) by a major clade (*c*. 1000 species) currently recognized as subfamily Gesnerioideae. Radiation of this group occurred in all biomes of tropical America and was accompanied by extensive phenotypic and ecological diversification. Here we performed phylogenetic analyses using DNA sequences from three plastid loci to reconstruct the evolutionary history of Gesnerioideae and to investigate its relationship with other lineages of Gesneriaceae and Lamiales. Our molecular data confirm the inclusion of the South Pacific Coronanthereae and the Old World (OW) monotypic genus *Titanotrichum* in Gesnerioideae and the sister-group relationship of this subfamily to the rest of the OW Gesneriaceae. Calceolariaceae and the NW genera *Peltanthera* and *Sanango* appeared successively sister to Gesneriaceae, whereas *Cubitanthus*, which has been previously assigned to Gesneriaceae, is shown to be related to Linderniaceae. Based on molecular dating and biogeographical reconstruction analyses, we suggest that ancestors of Gesneriaceae originated in South America during the Late Cretaceous. Distribution of Gesneriaceae in the Palaeotropics and Australasia was inferred as resulting from two independent long-distance dispersals during the Eocene and Oligocene, respectively. In a short time span starting at 34 Mya, ancestors of Gesnerioideae colonized several Neotropical regions including the tropical Andes, Brazilian Atlantic forest, cerrado, Central America and the West Indies. Subsequent diversification within these areas occurred largely *in situ* and was particularly extensive in the mountainous systems of the Andes, Central America and the Brazilian Atlantic forest. Only two radiations account for 90% of the diversity of Gesneriaceae in the Brazilian Atlantic forest, whereas half of the species richness in the northern Andes and Central America originated during the last 10 Myr from a single radiation. *Botanical Journal of the Linnean Society*, 2013, **171**, 61–79.

ADDITIONAL KEYWORDS: Andes – Australasia – Brazilian Atlantic forest – dispersal – historical biogeography – Lamiales – molecular dating – Neotropics – phylogeny – radiation.

INTRODUCTION

Gesneriaceae (150–160 genera, *c*. 3200 species) are a clade of perennial herbs, shrubs or small trees that display an outstanding morphological diversity related to the colonization of a wide range of habitats and the evolution of specialized plant–animal interactions (Wiehler, 1983; Weber, 2004; Weber & Skog, 2007 onwards). Gesneriaceae are particularly abundant at mid-elevation in the mountain forests where they grow as epiphytes or on rock outcrops. Gesneriaceae are comparatively less frequent in the lowlands where they predominantly occur on slopes, riverbanks and moist rocks in rainforest. Flowers of Gesneriaceae are highly diversified, reflecting adaptation to a wide range of pollinators including birds,

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bats and several types of bees (Sanmartin-Gajardo & Sazima, 2004, 2005; Marten-Rodriguez, Almarales-Castro & Fenster, 2009; Sanmartin-Gajardo & Vianna, 2010). Fruits can be either dry and dehiscent with seeds dispersed by wind or rain drops, or fleshy with seed dispersal relying mainly on animals such as birds or ants (Kleinfeldt, 1978; Wiehler, 1983; Davidson, 1988; Smith, 2001). This extensive morphological diversification coupled with high levels of convergence complicated the assessment of relationships in Gesneriaceae and caused considerable confusion in the early taxonomy of this family (Clark *et al.*, 2012).

Current phylogenetic evidence supports the division of Gesneriaceae into two main lineages. The first corresponds to subfamily Didymocarpoideae with *c.* 1900 species distributed in Africa, Eurasia and the Pacific (Weber, 2004; Möller *et al.*, 2009). The second is subfamily Gesnerioideae with > 1000 species all restricted to the Neotropics with the exception of tribe Coronanthereae (20 species; e.g. Salinas & Armesto, 2012), presenting a disjunct distribution in the temperate Andes (*Asteranthera* Hanst., *Mitraria* Cav. and *Sarmienta* Ruiz & Pav.) and Australasia (e.g. *Coronanthera* C.B.Clarke, *Fieldia* Cunn. and *Rhabdothamnus* Cunn.). The close relationship between Coronanthereae and Gesnerioideae was first proposed based on shared features in the development of their cotyledons (Burtt, 1963). Since then, this relationship has been repeatedly confirmed by molecular data (Smith, 1996; Mayer *et al.*, 2003; Möller *et al.*, 2009; Woo *et al.*, 2011). At a lower taxonomic level, continuing efforts to resolve phylogenetic relationships in Gesneriaceae have contributed to redefine the boundaries of many genera and tribes (Zimmer *et al.*, 2002; Perret *et al.*, 2003; Roalson *et al.*, 2005b; Clark *et al.*, 2006, 2012; Möller *et al.*, 2009). However, no phylogenetic analysis including all major lineages of Gesneriaceae has yet been performed and there is still no firm consensus on the affinities of taxa such as *Cubitanthus* Barringer, *Peltanthera* Benth., *Sanango* Bunting & Duke, *Titanotrichum* Soler. and Calceolariaceae with Gesneriaceae (Weber, 2004).

Several hypotheses exist for the age and place of origin of Gesneriaceae. According to Raven & Axelrod (1974), Gesneriaceae probably existed when Africa and South America were in closer contact, perhaps in the early Palaeogene (> 65 Mya), whereas the major groups of the family differentiated later. Building on this hypothesis, Burtt (1998) suggested that Coronanthereae could be the relict of a Gondwanan lineage, which further gave rise to both Old World (OW) Didymocarpoideae and New World (NW) Gesnerioideae. The alternative hypothesis is that the transoceanic distribution of Gesneriaceae post-dated the break-up of Gondwana and resulted from long-distance dispersal events following the southern hemisphere route described for plants (Morley, 2003; Sanmartín & Ronquist, 2004; Renner, 2005).

Discriminating between these hypotheses requires a robust time-calibrated phylogenetic framework. Such a result however is difficult to obtain for Gesneriaceae due to the lack of known fossils attributed to this family. In such a case, the extension of the phylogenetic analyses to a broader taxonomic context coupled with the use of relaxed molecular clock methods (Sanderson, 1997, 2002; Drummond *et al.*, 2006) can help estimate divergence time on nodes without fossils. Using this approach across the asterids, Bremer, Friis & Bremer (2004) found that Gesneriaceae have a crown age of 71 Mya and diverged early in Lamiales. Although this age estimate supports the hypothesis of a Palaeogene origin of the family as proposed by Raven & Axelrod (1974), a recent molecular dating of tribe Coronanthereae suggests that the origin of this group could largely post-date the separation of southern hemisphere landmasses, supporting the role of long-distance dispersal over Gondwanan vicariance for explaining the occurrence of Coronanthereae in both South America and Australasia (Woo *et al.*, 2011). Despite these breakthroughs, the lack of a global phylogenetic analysis for the family has so far prevented resolution of the biogeographical origin of Gesneriaceae as a whole.

Within the Neotropics, Gesneriaeceae show an Andean-centred distribution with the highest species richness in the northern Andes and Central America, and secondary centres of diversity in the West Indies (Skog, 1976; Marten-Rodriguez *et al.*, 2010) and the Brazilian Atlantic forest (Chautems *et al.*, 2005; Perret, Chautems & Spichiger, 2006) (Fig. 1). According to Gentry's (1982) survey, such Andean-centred taxa may represent one-third of all Neotropical plant species and account for the extraordinary biodiversity found in the Neotropics compared with other tropical regions. To date, few studies have investigated the genesis of this pattern (Pirie *et al.*, 2006; Antonelli *et al.*, 2009; Givnish *et al.*, 2011), despite its importance for identifying the biotic or abiotic factors that may have shaped the spatial distribution of diversity we see today (Antonelli & Sanmartín, 2011). In the NW Gesnerioideae, the hypothesis that the Andes may correspond to a centre of origin and a major source area of dispersals into other Neotropical regions still needs to be tested. Roalson, Skog & Zimmer (2008) indicated that ancestors of tribe Gloxinieae, and probably of Gesnerioideae as a whole, could be either widespread through Central America and western/Andean South America or restricted to Central America. This study also pointed to an early colonization of Central America and the Caribbean region at least 26 Mya, prior to the diversification of

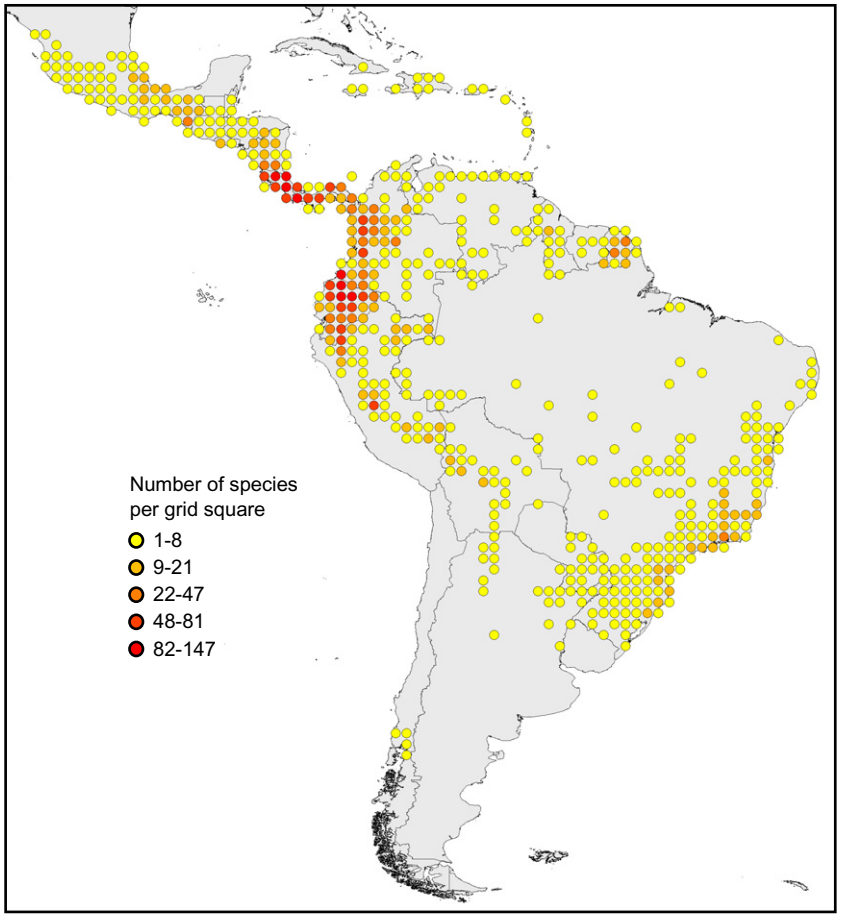

Figure 1. Distribution and species richness of Gesneriaceae in the New World based on 48 000 occurrences compiled from the GBIF data portal (http://data.gbif.org, November 2011), Perret *et al*. (2006) and unpublished data (A. Chautems). Note the Andean-centred distribution with a concentration of species in the northern Andes and Central America and the secondary centre of diversity in the Brazilian Atlantic forest. Occurrences in the temperate Andes of Chile correspond to species of Coronanthereae.

Gloxinieae in the Andes. This scenario suggests a more complex pattern than the one implying unidirectional movements between the Andes and the other surrounding biomes. However, additional data are needed to identify the geographical origin of Gesnerioideae and explore the temporal and spatial context of its spread in the Neotropics further.

Here we present a phylogenetic analysis based on nucleotide variation in three plastid loci (*matK*, *rps16* and *trnL-trnF*) and a sampling that includes all major lineages of Gesneriaceae with a particular emphasis on Neotropical taxa (198 species). To allow calibration of the resulting phylogenetic tree, we expanded this alignment using the dataset of Schäferhoff *et al*. (2010) to include representatives of all main lineages of Lamiales and the few known taxa with a fossil record. We produced a calibrated tree using a relaxed molecular clock approach (Drummond *et al*., 2006), and the biogeographical history of the family was inferred using a maximum-likelihood method that implements the dispersal–extinction–cladogenesis model (Ree *et al*., 2005; Ree & Smith, 2008). The aims of these analyses were (1) to resolve early branching relationships in Gesneriaceae and identify the closest related lineages of Lamiales, (2) to determine the geographical origin of Gesneriaceae, (3) to test whether the timing of the cladogenesis is consistent with a Gondwanan origin of the family or long-distance dispersals across the southern hemisphere and (4) to propose a biogeographical scenario that explains the current distribution of Gesneriaceae in the Neotropics.

MATERIAL AND METHODS

Taxon sampling and molecular markers

The sampling strategy was designed to encompass the majority of the lineages of NW Gesneriaceae and to

allow testing their phylogenetic position in the broader taxonomic context of Gesneriaceae and Lamiales. To achieve this goal, we sampled a total of 303 accessions representing 202 species of Gesneriaceae, 94 taxa from other families of Lamiales, and two outgroups from Solanaceae and one from Rubiaceae. Sampling in Gesneriaceae represents 194 species of Gesnerioideae, four species of Coronanthereae and four species of OW Didymocarpoideae (*sensu* Burtt & Wiehler, 1995). Taxa included in this study represent 37 of the 59 recognized genera in Gesnerioideae and > 80% of the Brazilian species (Araujo & Chautems, 2010). Genera not represented in this study represent < 9% of all described species in NW Gesneriaceae (Skog & Boggan, 2006). Monotypic genera with uncertain affinities in Gesneriaceae such as *Cubitanthus*, *Peltanthera*, *Sanango* and *Titanotrichum* were also included in this study.

Sequences of *matK*, *rps16* and *trnL-trnF* were obtained for most species of Gesneriaceae. These sequences were newly acquired for this study or gathered from published data (Perret *et al*., 2003; Araujo, Souza & Perret, 2010). Sequences for taxa of Lamiales other than Gesneriaceae were mainly obtained from the dataset of Schäferhoff *et al*. (2010) that includes sequences for 98 taxa representing all 23 families accepted in this order (APG III, 2009). Taxonomic sampling of this original dataset was slightly modified by pruning four taxa of Lentibulariaceae (*Utricularia subulata* L., *U. biloba* R.Br., *Pinguicula alpina* L. and *P. lusitanica* L.) to keep only one species per genus and by adding sequences for *Ligustrum* L. (HM751208, AF225257, AF231848) and *Fraxinus* L. (AM933427, AF225238, AF231830). Species names, voucher information and GenBank accession numbers for sequences used in this study are provided in Schäferhoff *et al*. (2010) and in Supporting Information, Table S1.

DNA sequencing

Total DNA was isolated from fresh or silica-gel dried leaf tissue using the NucleoSpin Plant II (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. The *rps16* intron and the *trnL-trnF* region were amplified and sequenced using primer pairs rpsF/rpsR2 and c/f, respectively (Taberlet *et al*., 1991; Oxelman, Liden & Berglund, 1997). A fragment of *c*. 1.8 kb including the entire coding region of *matK* and a portion of the 3′ *trnK* intron was first amplified and sequenced using the primer matK1F and matK1R for a restricted number of samples (Sang, Crawford & Stuessy, 1997). The resulting sequences were then used to design two internal primers specific for Neotropical Gesnerioideae, matK762F (5′-AACGTCTT GTGAAYGTCTTTGTT-3′) and matK943R (5′-CCTTC TTGGATGAAACCACAGC-3′), which were used as additional primers for sequencing. An alternative approach for *matK*, which turned out to be more successful, was to amplify and sequence two overlapping fragments using the pairs of primers matK1F/matK943R and matK762F/matK1R.

Amplification of selected regions was achieved in a 20-μL reaction mixture containing 2 μL of 10× PCR buffer, 2 μL of 25 mM $MgCl_2$, 0.4 μL of 10 mM dNTPs, 0.2 μL 5% bovine serum albumin, 1 μL each of 10 μM primer, 0.15 μL FastStart Taq DNA polymerase (5 U μL^{-1}) (Roche Diagnostics, Rotkreuz, Switzerland) and 12.25 μL double distilled H_2O. PCR was performed in a Biometra T3 thermocycler. Initial denaturation was programmed for 4 min at 95 °C, followed by 30 cycles at 94 °C for 45 s, 52 °C for 45 s and 72 °C for 1 min, plus a final extension of 7 min at 72 °C. PCR products were purified using NucleoFast© plates (Macherey-Nagel). Both stands were sequenced using BigDye™ Terminators (v2.0, Applied Biosystems, Inc., Warrington, UK). Cycle sequencing reactions were purified by ethanol precipitation and run on an ABI 377 sequencer following the manufacturer's protocols (Applied Biosystems). The program Sequencher 4.7 (Gene Code Corp., Ann Arbor, MI, USA) was used to edit and assemble complementary strands. Newly acquired sequences have been deposited in GenBank (supporting Table S1).

Phylogenetic and dating analyses

The three plastid regions (coding region of *matK*, *rps16* intron and *trnL-trnF* intron and spacer) were initially aligned with Muscle (Edgar, 2004) and then imported in MacClade 4 (Maddison & Maddison, 2003) for verification and manual adjustment. Regions of uncertain homology in *trnL-trnF* (positions 2822–2848) and the *rps16* intron (positions 4751–4801), totalling 80 characters, were excluded from the dataset. Sequences of the 3′ *trnK* intron were only partial and thus this region was excluded from the analyses, in contrast to Schäferhoff *et al*. (2010). Insertions/deletions (indels) were not coded as separate characters.

Divergence time analyses were performed using the program BEAST (Drummond & Rambaut, 2007). The four reference fossils of Lamiales and the two secondary calibration points used in the analyses are described in Table 1. All fossils selected in this study present diagnostic characters in their reproductive structures that enable their assignment to modern taxa at the generic or family level. The stem nodes to which these fossils were attached are indicated on the phylogenetic tree (supporting Fig. S1). The analyses were run for 50 million generations using a lognormal relaxed molecular clock. The model of DNA evolution

Table 1. Fossil records and secondary calibration points used in BEAST analyses. These calibration points are indicated on the phylogentic tree in Supporting Information Figure S1

Calibration point	Fossil/node	Node age (Mya)	Prior distribution	Mean/SD (offset) of lognormal distribution	References
A	Fossil fruits of *Fraxinus*	37	lognormal	2.5/0.5 (35.0)	Call & Dilcher (1992)
B	Fossil fruits of *Paulownia*	16–23	lognormal	1.5/0.5 (15.0)	Butzmann & Fischer (1997); Fischer & Butzmann (2006); Manchester *et al*. (2009)
C	Fossil seed of *Byblis*	37.2–48.6	lognormal	2.0/0.5 (35.0)	Conran & Christophel (2004)
D	Fossil fruits and seeds of Bignoniaceae	49.5	lognormal	1.5/0.5 (45.0)	Wesley & Hopkins (1994); Pigg & Wehr (2002)
E	Gesneriaceae/*Peltanthera* split	71	lognormal	2.5/0.5 (70.0)	Bremer *et al*. (2004)
F	Lamiales stem age	104–106	lognormal	2.5/1.0 (95.0)	Bremer *et al*. (2004); Janssens *et al*. (2009)

was set to GTR + Γ after likelihood test comparisons and the parameters of the model were estimated separately for each plastid region. Prior distributions for each calibration point are described in Table 1. A lognormal prior was used for each calibration point. This type of prior was selected because it gives high probabilities to dates close to the minimum age provided by the fossil calibration without restricting the possibility for much older dates through its heavy tail. We further decided to offset the distribution to move the minimum age possible with each lognormal distribution to be at the younger end of the fossil minimum age confidence interval. A Yule prior was set for the tree topology and all the remaining priors on other parameters were left to their default values. Two independent Markov chain Monte Carlo (MCMC) runs were performed by sampling trees and parameters every 1000th generation. Convergence of the two MCMC analyses was assessed using the program Tracer (Drummond & Rambaut, 2007). For each analysis, we specifically checked the effective sample size of each parameter and any asymptotic behaviour of the likelihood trace. We further compared the correlation of posterior distributions for each parameter estimated by the two independent analyses.

The use of fossil calibrations in divergence-time estimation is not straightforward because of the difficulty of reliably assigning fossils to specific nodes of a phylogenetic tree containing only extant species. We therefore performed a cross-validation procedure to assess the congruence between the calibration points used (Near & Sanderson, 2004). The divergence time analyses were repeated using the same settings as described above but by leaving out one calibration point at a time. A calibration was considered congruent if the mean value of its prior (Table 1) was contained within the 95% of its posterior distribution obtained by the BEAST analyses performed without constraining this calibration point.

Biogeographical analyses

Ranges of terminal taxa were categorized into seven eco-geographical regions, defined to reflect extant patterns of distribution in Gesneriaceae and the geological history of those areas (Antonelli *et al*., 2009; Santos *et al*., 2009; Hoorn *et al*., 2010). These regions are: (A) tropical Andes > 1000 m, (B) Brazilian Atlantic forest, in its broad definition (Oliveira & Fontes, 2000), (E) cerrado, (T) the temperate Andes in Chile and Argentina, (Z) Amazonian rainforest including lowland Amazonia, eastern foothills of the Andes < 1000 m, Guiana shield and northern Venezuela–Colombia, (C) Central America and (W) West Indies. Taxa ranging outside the American continents were assigned to either (O) Old world (Africa, Europe and tropical Asia), or (P) south-west Pacific (Australia, New Caledonia, New Zealand and Lord Howe Island). The Chocó, Guiana Highlands, caatingas and Chaco were not considered in this study. No Gesneriaceae endemic to the Chocó or Guiana highlands were sampled in this study and the few taxa occurring in the caatinga and Chaco were attributed to the Brazilian Atlantic forest, corresponding to the origin of these widespread taxa (Perret, Chautems & Spichiger, 2006). Biogeographical data for each species of Gesneriaceae included in the phylogenetic analyses were compiled from the World Checklist of Gesneriaceae (Skog & Boggan, 2011) and herbarium specimens. Species distributions were assigned to terminal branches except for the poorly sampled genera

Besleria L., *Napeanthus* Gardn., *Gasteranthus* Benth. and *Jovellana* Ruiz & Pav., for which terminals were scored to include their entire range. This approach aimed to consider the full geographical distribution for each genus, despite the risk of losing information important for the reconstruction of ancestral distributions. The range of each terminal taxon was defined by presence–absence coding (supporting Table S2).

Ancestral distributions were reconstructed on the 50% majority-rule consensus tree from the BEAST analysis using the dispersal–extinction–cladogenesis (DEC) model as implemented in the Lagrange software (Ree *et al*., 2005; Ree & Smith, 2008). Ancestral ranges were constrained to a maximum of two areas per node, which corresponds to the maximum observed for most extant species (except *Drymonia serrulata* Mart. and *Codonanthe uleana* Fritsch). To take into account the changing dispersal probabilities among areas through time, we constructed a stratified biogeographical model following the principles described in Ree & Smith (2008). We chose to divide our model into two time slices that reflect the main palaeogeographical changes during the history of Gesneriaceae, between 70 and 35 Mya, and between 35 Mya and the present day. For each time slice, scaling factors for the dispersal rate between America and other areas were selected according to Buerki *et al*. (2011). Colonization of the OW by Gesneriaceae was assumed to have started from tropical Southeast Asia (i.e. area D in Buerki *et al*., 2011) where the earliest branching lineage of OW Gesneriaceae is centred (i.e. Epithemateae; Mayer *et al*., 2003). In the Americas dispersal events between non-contiguous areas were scaled to a factor of 0.5 (i.e. C-B, C-E, C-T W-B, W-E, W-T). Among the seven phytogeographical units considered in the Americas, four were probably not present before 35 Mya. Therefore, dispersals from these areas were scaled to zero during the time slice between 70 and 35 Mya. These are the tropical Andes (A), Brazilian Atlantic forest (B), West Indies (W) and cerrado (E). Most evidence indicates that before 30 Mya only low hills occurred in the region today occupied by the northern and central Andes (Garzione *et al*., 2008; Hoorn *et al*., 2010). Likewise, the uplift of the mountain ranges in the Brazilian Atlantic forest such as the Serra do Mar and the Serra da Mantiqueira where Gesneriaceae occur today did not occur before the formation of the Continental Rift of south-eastern Brazil that started during the Oligocene (Riccomini *et al*., 1989; Hiruma *et al*., 2010). The origin of the West Indies could also post-date 35 Mya (Iturralde-Vinent & MacPhee, 1999), whereas the cerrado biome is probably not older than 10 Mya (Simon *et al*., 2009). The widely disjunct areas such as CB, CE, CT, WB, WE, WT and all pairs including areas from different continents (except PT and PO) were excluded from the analysis based on the biological implausibility of their spatial configuration. The python script used for the analysis was generated with the online configurator (http://www.reelab.net/lagrange/configurator/index). The palaeogeographical model and python script are available in the Supplementary Information (Figs S1 and S3).

RESULTS

Phylogenetic analyses

The combination of the three plastid regions resulted in an overall alignment of 300 taxa and 5746 characters including 2980 variable sites and 2146 potentially parsimony-informative characters.

The tree resulting from BEAST analysis is well resolved and most nodes defining the backbone of the phylogeny for Lamiales received maximum support (supporting Fig. S1). The topology is largely congruent with the inter-familial relationships found by Schäferhoff *et al*. (2010) with the main difference that our results place Byblidaceae as sister to Lentibulariaceae and provide further resolution in the clade including Lentibulariaceae, Verbenaceae, Acanthaceae, Bignoniaceae, Martyniaceae, Schlegeliaceae, Pedaliaceae, Thomandersiaceae (supporting Fig. S1). *Cubitanthus* is sister to *Stemodiopsis* Engl. at the base of Linderniaceae. Gesneriaceae and Calceolariaceae are sisters and form the first branch in core Lamiales (*sensu* Hilu *et al*., 2003; all Lamiales except Carlemanniaceae, Oleaceae, Plocospermataceae and Tetrachondraceae).

Relationships in Gesneriaceae are shown in Figure 2. *Peltanthera* and *Sanango* branch first in Gesneriaceae, the remainder of which divided into Didymocarpoideae and Gesnerioideae clades. In Gesnerioideae, tribes Napeantheae (including *Titanotrichum*), Beslerieae and the South Pacific Coronantheae constitute a basal grade. Monophyly of each of these clades is well supported [posterior probability (PP = 1)] but the relationships among them are defined by short and poorly supported branches (PP < 0.5). Remaining Gesnerioideae form a well-supported clade, core Gesnerioideae, which includes the monophyletic tribes Episcieae, Gesnerieae, Gloxinieae, Sinningieae and Sphaerorrhizeae. Although the monophyly of these clades is well supported (PP = 1), the relationships among them receive low support (PP < 0.5; Fig. 2).

Estimated ages of lineages

The two independent analyses performed with BEAST resulted in highly correlated posterior distri-

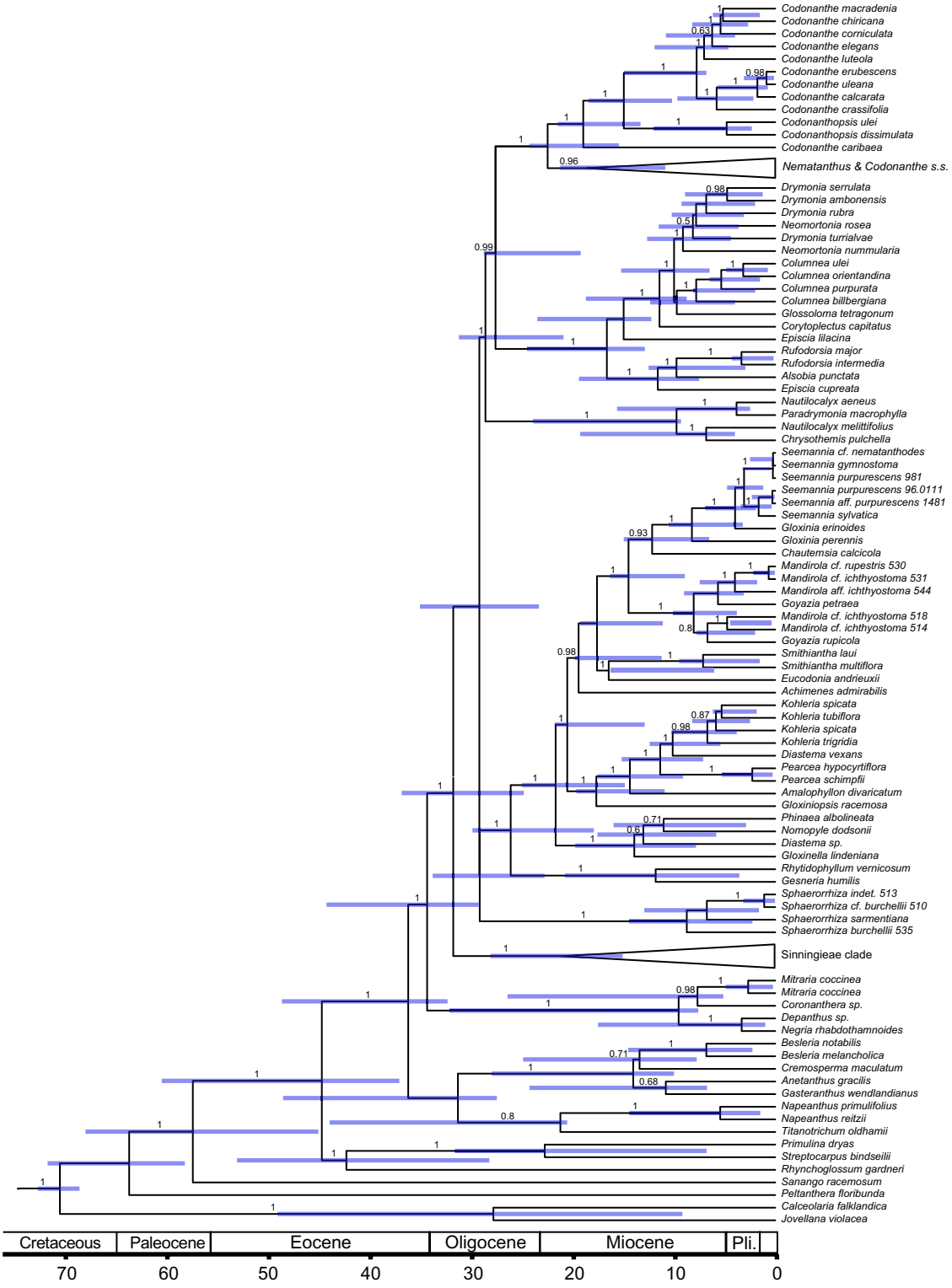

Figure 2. Chronogram of Gesneriaceae with 95% highest posterior density bars, based on BEAST analyses using four fossils and two secondary calibration points listed in Table 1. Bayesian posterior probabilities are indicated above branches when higher than 0.5. Geological time scale is shown at the bottom. The basal portion of the tree, including all other taxa of Lamiales, is available in supporting Figure S1. Detailed chronograms of Sinningieae and *Nematanthus*/*Codonanthe sensu stricto* clades are shown in supporting Figure S2.

Table 2. Stem and crown ages (in Mya) for major clades of Gesneriaceae obtained from BEAST analyses using priors described in Table 1; lower and upper 95% highest posterior densities are shown in parentheses

Clade	Stem age	Crown age
Gesneriaceae excluding *Sanango* and *Peltanthera*	57.5 (45.1, 68.1)	44.7 (37.1, 60.5)
Gesnerioideae	44.7 (37.1, 60.5)	36.2 (32.3, 48.7)
Coronanthereae	34.3 (32.3, 48.7)	9.5 (7.6, 32.2)
Core Gesnerioideae (Episcieae + Gesnerieae + Gloxinieae + Sinningieae + Sphaerorrhizeae)	34.3 (29.2, 44.3)	31.7 (24.8, 36.9)
Episcieae	29.2 (23.3, 35.1)	28.6 (20.9, 31.2)
Gesnerieae	26.1 (17.9, 29.9)	11.8 (3.5, 20.8)
Gloxinieae	26.1 (17.9, 29.9)	21.7 (14.8, 25.0)
Sinningieae	31.7 (24.8, 36.9)	21.1 (15.0, 28.1)
Sphaerorrhizeae	29.2 (22.8, 33.8)	8.7 (2.2, 14.4)

butions for each parameter. We therefore decided to merge these analyses after the removal of 20 million generations from each run as burn-in, based on the effective sample size and likelihood trace assignment. The cross-validation procedure to assess the congruence between the different calibration points showed that none of the fossils used was providing incongruent constraints (supporting Fig. S4). A calibration was considered congruent if the mean of its prior value (Table 1) was contained within the 95% of its posterior distribution obtained by the BEAST analyses performed without constraining this calibration point (supporting Fig. S4). In particular, the fossil calibration positioned in Lamiales, which was identified as problematic because of low support in the basal area of the tree (see Schäferhoff *et al*., 2010), did not provide incongruent divergence times (supporting Fig. S4). We therefore included all of them in the analyses. Results of our dating analyses are shown in Figure 2 with error bars indicating lower and upper 95% highest posterior densities. The estimated divergence times for Gesneriaceae were constrained at 71 Mya based on a secondary calibration point corresponding to the split between *Peltanthera* and the rest of Gesneriaceae (Table 1; Bremer *et al*., 2004). The 95% posterior probability of the dates on this node obtained after MCMC analyses, which includes the uncertainty of the tree reconstruction, the relaxed molecular clock and the model parameters, ranged from 68.6 to 72.8 Mya. This is the closest constraint that we could use for inferring divergence times in Gesneriaceae. It has a direct influence on the times estimated for the other nodes of the family listed in Table 2.

Ancestral area reconstruction

The history of the distribution of Gesneriaceae was inferred using DEC modelling. The results of our stratified model are presented on the 50% majority-rule consensus from the BEAST analysis in Figure 3. Reconstructions of ancestral areas indicate that ancestors of Gesneriaceae originated in South America, probably in an area comprising the temperate Andes and the Amazonian rainforest (Fig. 3). Following the Antarctic route, NW ancestors dispersed to the Palaeotropics giving rise to Didymocarpoideae and then, independently, to the South Pacific leading to the transoceanic distribution of Coronanthereae. Long-distance dispersal from NW to OW was also inferred along the branch leading to the OW monospecific genus *Titanotrichum* (Fig. 3).

Ancestors of Gesnerioideae dispersed to the Brazilian Atlantic forest (B), tropical Andes (A), cerrado (E), West Indies (W) and Central America (C) early in the history of the group (Fig. 3). Subsequent diversification within these areas occurred through different biogeographical patterns according to the lineage. Sinningieae, Sphaerorrhizeae and Gesnerieae diversified mainly *in situ* within their current area of distribution in the Brazilian Atlantic forest, cerrado and West Indies, respectively. Gloxinieae originally diversified in the tropical Andes from where they colonized Central America to the north and the cerrado to the south-east. Episcieae probably originated in the Amazonian rainforest and subsequently colonized the tropical Andes, Central America and the Brazilian Atlantic forest. Colonization of the tropical Andes and Central America was marked by an extensive diversification giving rise to the large genera

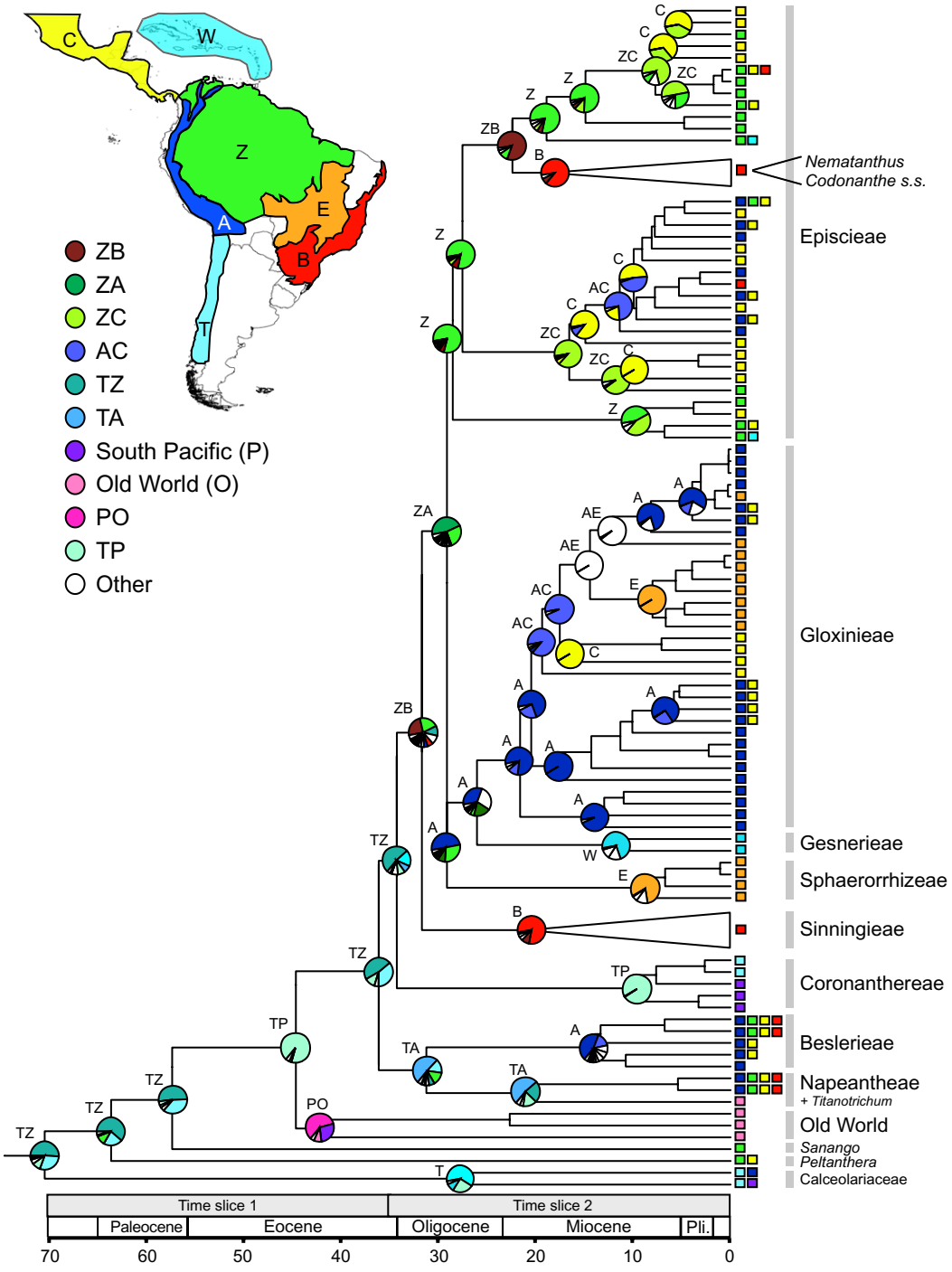

Figure 3. Maximum-likelihood reconstruction of geographical range evolution inferred by the Lagrange method based on a stratified biogeographical model plotted on the BEAST chronogram shown on Figure 2. Pie charts represent relative probabilities of ancestral ranges. Reconstruction with the highest relative probability is indicated next to each pie chart. Geological time scale is shown at the bottom, and major clades of Gesneriaceae are indicated on the right-hand side. Area abbreviations are as followed: A, Tropical Andean mountains (> 1000 m); B, Brazilian Atlantic forest; C, Central America; E, Cerrado; T, Temperate Andes; Z, Amazonian rainforest including lowland Amazonia, Sub-Andes, Guiana shield and northern Venezuela–Colombia; W, West Indies. Taxa occuring outside America were assigned to either (O) Old world (Africa, Europe and tropical Asia) or (P) south-west Pacific (Australia, New Caledonia, New Zealand and Lord Howe Island).

Columnea L., *Drymonia* Mart. and *Glossoloma* Hanst that altogether account for 60% of the species in Episcieae. Diversification in the other lineages of Episcieae occurred either across the Neotropical lowlands (e.g. *Nautilocalyx* Hanst., *Chrysothemis* Decne, *Paradrymonia* Hanst.), or in the Brazilian Atlantic forest [*Codonanthe* (Mart.) Hanst. and *Nematanthus* Schrad.].

DISCUSSION

Phylogenetic relationships

The resolution of relationships among families of Lamiales has been recently improved by the use of fast evolving plastid sequences and the sampling of all 23 families currently recognized in the order (Schäferhoff *et al*., 2010). The analysis of this dataset complemented by our samples is largely congruent with the original result of Schäferhoff *et al*. (2010) (supporting Fig. S1). We confirm the sister relationship between Gesneriaceae and Calceolariaceae (Rahmanzadeh *et al*., 2005; Andersson, 2006; Schäferhoff *et al*., 2010). The main difference with previous analyses is the finding of a sister relationship between Lentibulariaceae and Byblidaceae (Supporting Information, Fig. S1), two families characterized by carnivory. This result indicates that a single origin of this particular feature in Lamiales may be more likely than the hypothesis of two independent origins proposed by Müller *et al*. (2004) and Schäferhoff *et al*. (2010).

The analysis of a large sampling in Lamiales and Gesneriaceae allowed testing the phylogenetic position of the monotypic genera *Cubitanthus*, *Peltanthera*, *Sanango* and *Titanotrichum*, of which affinities with Gesneriaceae have been considered unclear (Wiehler, 1976; Weber, 2004). Our phylogenetic tree clearly placed *Cubitanthus* in Linderniaceae (Supporting Information Fig. S1). The only species of this genus, *C. alatus* (Cham. & Schltdl.) Barringer, is endemic to the State of Bahia in Brazil (Chautems, 1991). *Cubitanthus* has been affiliated to Gesneriaceae mainly because parietal placentation, a commonly used diagnostic character for this family, was inferred from an illustration in the original description of this taxon (Barringer, 1984). A reinvestigation of this feature based on transverse sections of fresh fruit has, however, revealed that parietal placentation is restricted to the upper portion of the fruit, whereas the lower portion shows axile placentation (F. Stauffer, unpubl. data). This type of mixed placentation occurs in some taxa of Gesneriaceae, but is also present in several lineages previously classified in Scrophulariaceae *sensu lato* (Hartl, 1956; Weber, 1971). Therefore, ovary structure alone is not sufficient to characterize Gesneriaceae (Weber, 2004). In contrast, several morphological features of *Cubitanthus*, such as the four-winged stem, one-flowered axillary florescence and bilabiate corolla limb with entire upper lip and three-lobed lower lip, match morphological features that characterize Linderniaceae, reinforcing our phylogenetic results (Rahmanzadeh *et al*., 2005). In Linderniaceae, *Cubitanthus* is found to be closely related to *Stemodiopsis* Engl., a genus growing in rock crevices in tropical Africa and Madagascar (Fischer, 1997). However, further clarification of the relationship and taxonomy of *Cubitanthus* will require the sampling of other NW Linderniaceae [e.g. species of *Lindernia* All. and *Torenia* L. (Lewis, 2000)].

The monospecific genera *Peltanthera* and *Sanango* constitute the first branches at the base of Gesneriaceae (Fig. 2). *Peltanthera* is widely distributed at low altitudes along the eastern slope of the tropical Andes and in Central America up to Costa Rica, whereas *Sanango* is restricted to the sub-Andean region of Peru and south-eastern Ecuador (Bunting & Duke, 1961; Skog & Boggan, 2011). Both genera are small trees that have traditionally been placed in Loganiaceae and Buddlejaceae (now Scrophulariaceae), but *Sanango* was later transferred to Gesneriaceae by Wiehler (1994). Our results confirm this taxonomic decision and suggest that *Peltanthera* should be either transferred to Gesneriaceae or considered a separate family. This is in line with earlier molecular evidence and chemical data that suggest a close relationship of both genera with Gesneriaceae (Jensen, 1996, 2000; Smith *et al*., 1997; Oxelman, Backlund & Bremer, 1999; Bremer *et al*., 2002).

Gesneriaceae were next divided into two major radiations; one corresponds to the OW Gesneriaceae (Möller *et al*., 2009), the other to Gesnerioideae, which are restricted to the Neotropics except for the South Pacific Coronanthereae and the OW genus *Titanotrichum*. This early split into two major clades and the placement of Coronanthereae near the base of Gesnerioideae have been repeatedly supported by other molecular data (Smith, 1996; Mayer *et al*., 2003; Wang, Möller & Cronk, 2004a; Möller *et al*., 2009; Woo *et al*., 2011). Our finding of a sister relationship between Coronanthereae and Napeantheae/Beslerieae was only poorly supported, indicating that additional data are still needed to further assess relationships among early branching Gesnerioideae (Clark *et al*., 2010).

Titanotrichum appears to be related to *Napeanthus* (PP = 0.8), confirming the result of Wang *et al*. (2004a), who placed this monotypic genus among early branching Gesnerioideae. In contrast to its closest relatives, which are endemic to the Neotropics, *Titanotrichum* occurs in Taiwan, adjacent regions of

China and the Ryukyu Isands of Japan (Wang, Möller & Cronk, 2004b). Plants of *Titanotrichum* can reproduce vegetatively through the production of numerous small bulbils in the inflorescence (Wang & Cronk, 2003), a feature not seen in other Gesneriaceae. Because of this geographical and morphological distinctiveness, this species has been either classified in its own tribe in Didymocarpoideae (= Cyrtandroideae) (Burtt & Wiehler, 1995) or excluded from the family altogether (Weber, 2004). These new molecular data point to the need to include *Titanotrichum* in Gesnerioideae.

Tribes Episceae, Gesnerieae, Gloxinieae, Sinningieae and Sphaerorrhizeae form a well-supported clade, which includes 75% of species of Gesnerioideae (Weber, 2004; Skog & Boggan, 2006). These tribes are resolved as monophyletic entities with their current circumscriptions, reflecting the extensive taxonomic and phylogenetic efforts that have been spent to redefine monophyletic tribes in core Gesnerioideae (Wiehler, 1983; Smith, 1996; Zimmer *et al*., 2002; Perret *et al*., 2003; Roalson, Boggan & Skog, 2005a; Roalson *et al*., 2005b; Clark *et al*., 2006; Araujo *et al*., 2010). Relationships among these tribes are congruent with previous analyses based on nuclear and plastid sequences with the exception of Sphaerrohizeae, which forms a clade with Gloxinieae, in contrast to Zimmer *et al*. (2002), who placed it as a sister clade to Sinningieae. However, these inter-tribal relationships are poorly supported and additional data will be necessary to determine whether this conflict results from incongruence among datasets or is related to random effects arising from insufficient informative characters. With few exceptions, which will not be discussed here, relationships within Episceae, Gloxinieae and Sinningieae largely agree with previous studies focused on these groups (supporting Fig. S2; Perret *et al*., 2003; Roalson *et al*., 2005b; Clark *et al*., 2006, 2012; Araujo *et al*., 2010).

Origin and early history of Gesneriaceae

Ancestral range analyses and molecular dating indicate that ancestors of Gesneriaceae probably originated during the Late Cretaceous in South America (Fig. 3). Inference of this place of origin relies mainly on the placements of the NW taxa Calceolariaceae, *Sanango* and *Peltanthera* at the base of the Gesneriaceae, confirming previous results based on smaller taxonomic samples (Oxelman *et al*., 1999; Bremer *et al*., 2002; Wang *et al*., 2004a; Andersson, 2006; Schäferhoff *et al*., 2010). According to our biogeographical reconstruction, the cradle of Gesneriaceae could comprise both the temperate Andes and Amazonian rainforest. Inclusion of the temperate Andes in the ancestral area of Gesneriaceae relies on the reconstruction of a temperate Andean origin for Calceolariaceae, a result consistent with previous phylogenetic studies focused on this family (Andersson, 2006; Cosacov *et al*., 2009; Nylinder *et al*., 2012) and palaeogeographical evidence suggesting that mountains in the temperate Andes were already uplifted and subjected to a warm and equable climate (Taylor, 1991; Morley, 2003). On the other hand, the putative occurrence of ancestors of Gesneriaceae in the Amazonian rainforest is mainly explained by the current occurrence of the trees *Sanango* and *Peltanthera* at low elevations along the eastern foothills of the tropical Andes (Bunting & Duke, 1961).

The estimated age of the split between OW Didymocarpoideae and Gesnerioideae (44.7 Mya; Table 2) did not support the traditional hypothesis that major lineages of Gesneriaceae would have evolved in vicariance following the Cretaceous breakup of Gondwana (Burtt, 1998). The alternative hypothesis, involving interplate dispersal from South America to Australasia, is thus favoured to explain the origin of the transoceanic distribution of Gesneriaceae (Fig. 3). Although the mechanisms of dispersal in this family are poorly known, long-distance dispersal has been invoked to explain the scattered distribution of genera such as *Cyrtandra* J.R.Forst. & G.Forst., *Aeschynanthus* Jack and *Columnea* on islands throughout the Pacific or the Caribbean sea (Cronk *et al*., 2005; Clark *et al*., 2008; Roalson *et al*., 2008). According to our biogeographical reconstruction, a first dispersal of Gesneriaceae from the NW to the Palaeotropics could have occurred during the late Palaeocene/Early Eocene thermal maximum following the Antarctica route (Fig. 3). During this period the climate was sufficiently warm to allow floristic exchanges of megathermal plants between South America and Australasia via Antarctica (Morley, 2003). Examples of such dispersals have been reconstructed for several lineages in Malvaceae, Myrtaceae, Sapindaceae, Sapotaceae and *Nothofagus* Blume (Baum *et al*., 2004; Sytsma *et al*., 2004; Knapp *et al*., 2005; Bartish *et al*., 2011; Buerki *et al*., 2011).

The placement of Coronanthereae in Gesnerioideae supports a later dispersal event from South America to the south-west Pacific, in line with the recent results of Woo *et al*. (2011) demonstrating the NW origin of Coronanthereae (Fig. 3). The age of Coronanthereae was estimated at 34.3 Mya (Table 2) in good agreement with the 35.1 Mya found by Woo *et al*. (2011). This period corresponds to the separation of Australia from Antarctica and South America (35 Mya) followed by the opening of the Drake Passage further isolating southern South America from Antarctica (*c*. 30 Mya; Sanmartín & Ronquist, 2004). Direct overland connections or dispersals across the still narrow water gaps that divided these

landmasses could have allowed range extension of Coronantherae from southern South America, up to Australasia through Antarctica. The fossil record from the Oligocene (c. 34 Mya) in the Antarctic Peninsula and Ross Sea regions shows that vegetation was composed of angiosperms dominated by *Nothofagus*, analogous to the present-day Valdivian temperate forests that occur between 37° and 48°S in Chile (Thorn & DeConto, 2006). The current occurrence of Coronanthereae (i.e. *Asteranthera*, *Sarmienta* and *Mitraria*) in such vegetation in the northern part of Chiloé Island (42°30′S) in Chile (Fig. 1; Salinas, Arroyo & Armesto, 2010) suggests that ancestors of this lineage could have been hardy enough to tolerate environmental conditions in Antarctica during the Oligocene. It is therefore plausible that Coronanthereae dispersed from South America to Australasia via Antarctica in the same way hypothesized for other cool-tolerant taxa such as *Fuchsia* L., *Nothofagus*, *Ourisia* Juss. and the palm subfamily Ceroxyloideae (Berry *et al*., 2004; Knapp *et al*., 2005; Meudt & Simpson, 2006; Trenel *et al*., 2007).

Finally, the puzzling position of the OW *Titanotrichum* in the NW tribe Napeantheae among early branching Gesnerioideae suggests that *Titanotrichum* arose in Taiwan and China following long-distance dispersal from South America during the Miocene. This long-distance dispersal scenario may be supported by the evolution in *Titanotrichum* of a dispersal mechanism based on the production of tiny and numerous bulbils in the inflorescence (Wang & Cronk, 2003).

Historical biogeography in the New World

In our dating analyses, core Gesneroideae (i.e. Episcieae, Gesnerieae, Gloxinieae, Sinningieae and Sphaerorrhizeae), which together comprise 75% of the current diversity of NW Gesneriaceae, arose in the early Oligocene (34.3 Mya, Table 2). In a short time span during the Oligocene, ancestors of Gesnerioideae colonized most biogeographical areas in the Neotropics (Fig. 3). Although the early biogeographical pattern of this rapid extension is inferred with uncertainties, our results indicate different biogeographical patterns for each major clade.

The Sinningieae clade centred in the Brazilian Atlantic forest arose around 30 Mya probably from an early vicariant split with an ancestor from the Amazonian rainforest (Fig. 3). Subsequent diversification of Sinningieae occurred mostly *in situ* through allopatric speciation among and within the different phytogeographical units that compose this biodiversity hotspot (Perret *et al*., 2006, 2007). This radiation began in the context of an increase of aridity in eastern South America during the Oligocene and the early uplift of the Serra do Mar and Serra da Mantiqueira ranges after the formation of the Continental Rift of south-eastern Brazil (Almeida, 1976; Riccomini *et al*., 1989; Hiruma *et al*., 2010). The concomitant evolution of a water-storage organ, seasonal growth and saxicolous habit in this clade (Perret *et al*., 2003) could have facilitated the expansion and diversification of this group in a subtropical environment increasingly marked by rocky habitats. Adaptation to seasonality may also be linked to the expansion of these taxa into more inland habitats such as *campos rupestres* and semi-deciduous forests (Oliveira & Fontes, 2000) and their persistence through climatic changes that would have particularly impacted the length of the dry season in this region (Behling & Negrelle, 2001; Ledru *et al*., 2005). At the same time, niche conservatism and the ecological preferences of Sinningieae for rocky environments, together with the lack of such habitats in the surrounding areas (i.e. Chaco, cerrado and lowland Amazonia), could explain their rarity in these biomes and their long-term isolation in the mountain systems of the Atlantic forest.

Divergence between the Caribbean endemic Gesnerieae and Gloxinieae was estimated at 26.1 Mya (Table 2) in agreement with previous analysis by Roalson *et al*. (2008) and the hypothesis that the Greater Antilles/Aves Ridge land bridge (GAARlandia), connecting north-eastern South America and the Greater Antilles between 35 and 25 Mya may have facilitated biotic dispersal into the Caribbean region (Iturralde-Vinent & MacPhee, 1999). Ancestral area reconstruction indicates that Gloxinieae have a centre of origin in the tropical Andes and subsequently colonized Central America during the early Miocene. This result is not congruent with the reconstruction of Roalson *et al*. (2008) suggesting that the ancestral area of Gloxinieae could be Central America or both Central America and western South America. The reconstruction of a Central American origin for Gloxinieae was mainly derived from their finding of an early branching position for the taxa endemic to Central America and Mexico (i.e. *Achimenes* Pers., *Eucodonia* Hanst., *Moussonia* Regel, *Smithiantha* Kuntze and *Solenophora* Benth.), a result that is not supported in the present study.

The cerrado appears to have been colonized independently on two occasions from the tropical Andes, by tribe Sphaerorrhizeae and by the clade including *Mandirola* Decne. and *Goyazia* Taub. in Gloxinieae (Fig. 3). Although cerrado and Atlantic forest are neighbouring areas, we did not find a close relationship between the taxa endemic to these biomes. Sphaerorrhizeae probably diverged early in the history of Gesnerioideae at 29.2 Mya (Table 2), whereas the *Mandirola*/*Goyazia* clade originated later in the mid-Miocene (*c*.15 Mya; Fig. 2). Despite

the large difference in the stem age of these clades, their crown ages were both dated around 10 Mya (Fig. 2), a period that coincides with the probable origin of cerrado according to fossil records and the molecular dating of lineages endemic to this biome (Pennington, Lewis & Ratter, 2006; Simon *et al*., 2009).

The origin of the species-rich clade Episcieae (477 species) was inferred in the Amazonian rainforest. This result should be considered with caution, however, because of the limited number of species sampled in this clade and the distribution of some taxa across a wide range of elevations on the Andean flanks. Despite this limitation, our data are consistent with an early divergence of Episcieae into three main clades that show distinct biogeographical patterns and habitat preferences.

The earliest diverging clade in Episcieae is formed by the genera *Chrysothemis*, *Nautilocalyx* and *Paradrymonia* (Fig. 2). Monophyly of this clade and its sister relationship with the rest of Episcieae have been recovered in other studies based on more extensive taxon sampling (Clark *et al*., 2006, 2012). This clade includes terrestrial or semi-epiphytic species growing in the understory of tropical rainforest throughout most of the Neotropical lowlands (Wiehler, 1978; Skog, 1979; Kvist *et al*., 2005; Feuillet, 2009). Our reconstruction suggests an origin of this lineage in the Amazonian rainforest or in a region combining both Amazonia and Central America (Fig. 3). This geographical origin and an ecological preference for terrestrial habitat are only shared with other basal lineages endemic to the Guyana shield (i.e. *Cremersia* Feuillet & L.E.Skog, *Lembocarpus* Leeuwenb., *Rhoogeton* Leeuwenb. and some species of *Episcia* Mart; Clark *et al*., 2012), whereas most of the remaining Episcieae are epiphytic and highly diverse in montane forests.

The second major clade of Episcieae is composed of the epiphytic genera *Codonanthe*, *Codonanthopsis* and *Nematanthus* (Fig. 2), in agreement with previous phylogenetic analyses (Clark & Zimmer, 2003; Clark *et al*., 2006, 2012). Our biogeographical reconstructions indicate a widespread origin of this lineage across South American rainforests followed by an early divergence between a Brazilian Atlantic forest and an Amazonian clade, dating back to the Oligocene/Miocene boundary (*c*. 23 Mya; Fig. 3). After this initial split, Amazonian ancestors of *Codonanthe* further colonized Central America during the Middle Miocene expanding the range of this lineage to a vast area including most of northern South America and Central America (Fig. 3). By contrast, diversification in the sister clade comprising the rest of the *Codonanthe* and *Nematanthus* was restricted to the Brazilian Atlantic forest, without any further range expansion into the Amazonian rainforest, even during the wet periods of the Quaternary that could have allowed expansion of gallery forest and biotic exchanges between these areas (Costa, 2003; Wang *et al*., 2004). This observation and the current absence of *Codonanthe* and *Nematanthus* in the cerrado and caatinga suggest that the spread of a more open vegetation along a dry corridor separating the Amazonian and Atlantic rainforest has constituted an efficient barrier to dispersal in this group of plants from the early Miocene to the present day. The finding of a similar vicariance in a forest-restricted group of bufonid toads (*Dendrophryniscus*) dating back to the Eocene (Fouquet *et al*., 2012) also points to the role of habitat barriers in the genesis and persistence of disjunct distributions between Amazonian and Atlantic rainforest. However, studies in more plant groups will be necessary to assess further how such deep divergences have contributed to the floristic distinctiveness of the Brazilian Atlantic forest (Santos *et al*., 2007; Fiaschi & Pirani, 2009).

The third major clade of Episceae comprises taxa that have extensively radiated in the montane areas of Central America and tropical Andes. According to the reconstruction in this clade, colonization of tropical Andes probably occured around 15 Mya from Central America (Fig. 3). Subsequent diversification in this clade could have involved repeated episodes of vicariance and range expansion between the northern Andes and Central America (Fig. 3). However, resolving the intricate biogeographical relationships between these two areas will require a more exhaustive species sampling. Diversification in this lineage was particularly high during the last 10 Myr with the origin of species-rich genera *Columnea* (*c*. 200 spp.), *Drymonia* (*c*. 70 spp.) and *Glossoloma* Hanst. (27 spp.), which form a monophyletic group (Fig. 2) in agreement with previous results (Clark *et al*., 2006, 2012). Evolution of this clade coincides with intense periods of Andean mountain building that had begun by the end of the Middle Miocene (*c*. 12 Mya; Hoorn *et al*., 2010; Mora *et al*., 2010). Taken together, these three genera account for *c*. 60% of the species diversity in Episcieae (Skog & Boggan, 2006) and 45–50% of the species of Gesneriaceae occurring in Ecuador (Skog, 1999) and Panama (Skog, 1979). This single radiation thus largely accounts for the higher richness of Gesneriaceae in the northern Andes and Central America compared with any other Neotropical area (Fig. 1).

CONCLUSIONS AND PERSPECTIVES

This study provides the first time-calibrated phylogenetic tree including all main lineages of Gesneriaceae and Lamiales. The result was used in combination

with distribution data to reconstruct the biogeographical history of Gesneriaceae, with particular focus on the Neotropics. Our divergence time estimate between the OW Didymocarpoideae and the Gesnerioideae clade, dated at 44.7 Mya, does not corroborate the role of Gondwanan vicariance in explaining the pantropical distribution of Gesneriaceae. Instead, we propose that ancestors of Gesneriaceae first originated in the NW and then independently colonized the Palaeotropics and Australasia during the Eocene and Oligocene, probably by means of long-distance dispersals across the southern hemisphere. This hypothesis is in agreement with the results of Woo *et al*. (2011) for Coronanthereae.

In the Neotropics, the Gesnerioideae radiation started during the Oligocene with a rapid range expansion into every biome currently occupied by this plant group. Patterns of dispersal and vicariance between biomes were congruent, at least on a broad scale, with the geological and climatic events that have shaped the landscape of the Neotropics. Species richness in biomes resulted from *in situ* diversification of a limited number of radiations. In particular, we found that only two endemic radiations (*Codonanthe*/*Nematanthus* and Sinningieae) from the early Miocene account for almost 90% of the diversity of Gesneriaceae in the Brazilian Atlantic forest (Chautems, 2009), whereas *c*. 50% of the species richness in the tropical Andes and Central America areas may have resulted from a single radiation that took place in the past 10 Myr, during the most intense period of Andean orogeny (Hoorn *et al*., 2010; Mora *et al*., 2010). These observations are in line with other studies suggesting that plant diversity in the Neotropics could have arisen from a combination of early Miocene (mature) radiations and more recent bursts of diversification (Pennington *et al*., 2004; Linder, 2008).

Although this study provides new insights into the biogeographical history of Gesneriaceae, it also points to the need to investigate biogeographical patterns in areas of high diversity at a finer scale. A full understanding of the origin of diversity across western Amazonia, the Andean foothills, the Andean Cordillera and Central America will require a better clarification of the biogeographical movements between these regions. Recent and ongoing efforts to resolve phylogenetic relationships in the species-rich tribe Episcieae could provide the necessary framework to address this issue (e.g. Clark *et al*., 2012).

Increasing the taxon sampling of this study may help further identify factors associated with diversification in Gesnerioideae. The description of the temporal dynamics of species diversification and its correlation with the colonization of new areas or with the evolution of morphological novelties is now possible owing to recent methodological breakthroughs (e.g. Moore & Donoghue, 2007; Silvestro, Schnitzler & Zizka, 2011). Gentry (1982) observed that Andean-centred plant groups, which have extensively radiated in the Neotropics, tend to present similar ecological features including hummingbird pollination and epiphytism. When these traits evolved and how they interplay with geographical movements to influence diversification rates, however, is little known. The high frequency of hummingbird pollination and epiphytism in Gesnerioideae and the independent evolution of these traits among distinct lineages and geographical areas make this plant group particularly appropriate to address these questions.

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REFERENCES

Almeida FFMD. 1976. System of continental rifts bordering Santos Basin, Brazil. *Anais Da Academia Brasileira de Ciencias* **48:** 15–26.

Andersson S. 2006. On the phylogeny of the genus *Calceolaria* (Calceolariaceae) as inferred from ITS and plastid *matK* sequences. *Taxon* **55:** 125–137.

Antonelli A, Nylander JAA, Persson C, Sanmartín I. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences of the United States of America* **106:** 9749–9754.

Antonelli A, Sanmartín I. 2011. Why are there so many plant species in the Neotropics? *Taxon* **60:** 403–414.

APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161:** 105–121.

Araujo AO, Chautems A. 2010. *Gesneriaceae*. In: Forzza RC, Leitman PM, Costa AF, Carvalho AA Jr., Peixoto AL, Walter BMT, Bicudo C, Zappi D, Costa DP, Lleras E, Martinelli G, Lima HC, Prado J, Stehmann JR, Baumgratz JFA, Pirani JR, Sylvestre L, Maia LC, Lohmann LG, Queiroz LP, Silveira M, Coelho MN, Mamede MC, Bastos MNC, Morim MP, Barbosa MR, Menezes M, Hopkins M, Secco R, Cavalcanti TB, Souza VC, eds. *Lista de Espécies da Flora do Brasil*. Jardim Botânico do Rio de Janeiro. Available at: http://floradobrasil.jbrj.gov.br/2010/FB000119

Araujo AO, Souza VC, Perret M. 2010. *Chautemsia calcicola*: a new genus and species of Gloxinieae (Gesneriaceae) from Minas Gerais, Brazil. *Taxon* **59:** 203–208.

Barringer K. 1984. *Cubitanthus*, a new genus of Gesneriaceae from Brazil. *Journal of the Arnold Arboretum* **65:** 145–147.

Bartish IV, Antonelli A, Richardson JE, Swenson U. 2011. Vicariance or long-distance dispersal: historical biogeography of the pantropical subfamily Chrysophylloideae (Sapotaceae). *Journal of Biogeography* **38:** 177–190.

Baum DA, Smith SD, Yen A, Alverson WS, Nyffeler R, Whitlock BA, Oldham RL. 2004. Phylogenetic relationships of Malvatheca (Bombacoideae and Malvoideae; Malvaceae *sensu lato*) as inferred from plastid DNA sequences. *American Journal of Botany* **91:** 1863–1871.

Behling H, Negrelle RRB. 2001. Tropical rain forest and climate dynamics of the Atlantic lowland, Southern Brazil, during the late Quaternary. *Quaternary Research* **56:** 383–389.

Berry PE, Hahn WJ, Sytsma KJ, Hall JC, Mast A. 2004. Phylogenetic relationships and biogeography of *Fuchsia* (Onagraceae) based on noncoding nuclear and chloroplast DNA data. *American Journal of Botany* **91:** 601–614.

Bremer B, Bremer K, Heidari N, Erixon P, Olmstead RG, Anderberg AA, Kallersjo M, Barkhordarian E. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molecular Phylogenetics and Evolution* **24:** 274–301.

Bremer K, Friis EM, Bremer B. 2004. Molecular phylogenetic dating of asterid flowering plants shows early Cretaceous diversification. *Systematic Biology* **53:** 496–505.

Buerki S, Forest F, Alvarez N, Nylander JAA, Arrigo N, Sanmartín I. 2011. An evaluation of new parsimony-based versus parametric inference methods in biogeography: a case study using the globally distributed plant family Sapindaceae. *Journal of Biogeography* **38:** 531–550.

Bunting GS, Duke JA. 1961. *Sanango*: new Amazonian genus of Loganiaceae. *Annals of the Missouri Botanical Garden* **48:** 269–274.

Burtt BL. 1963. Studies in the Gesneriaceae of Old World. XXIV. Tentative keys to the tribes and genera. *Notes from the Royal Botanic Garden Edinburgh* **24:** 205–220.

Burtt BL. 1998. Climatic accommodation and phytogeography of the Gesneriaceae of the Old World. In: Mathew P, Sivadasan M, eds. *Diversity and taxonomy of tropical flowering plants*. Calicut: Mentor Books, 1–27.

Burtt BL, Wiehler H. 1995. Classification of the family Gesneriaceae. *Gesneriana* **1:** 1–4.

Butzmann R, Fischer TC. 1997. Description of the fossil fruit *Paulownia inopinata* nov. spec. from the Middle Miocene of Unterwohlbach (Bavaria) and other possible occurences of the genus in the Tertiary. *Documenta Naturae* **115:** 1–13.

Call VB, Dilcher DL. 1992. Investigations of angiosperms from the Eocene of southeastern North-America – samaras of *Fraxinus wilcoxiana* Berry. *Review of Palaeobotany and Palynology* **74:** 249–266.

Chautems A. 1991. A família Gesneriaceae na região cacaueira do Brasil. *Revista Brasileira de Botânica* **14:** 51–59.

Chautems A. 2009. Gesneriaceae. In: Stehmann JR, Forzza RC, Salino A, Sobral M, Costa DP, Kamino LHY, eds. *Plantas da floresta Atlântica*. Rio de Janeiro: Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, 285–288.

Chautems A, Lopes TCC, Peixoto M, Rossini J. 2005. Five new species of *Nematanthus* Schrad. (Gesneriaceae) from eastern Brazil and a revised key to the genus. *Selbyana* **25:** 210–224.

Clark JL, Funke MM, Duffy AM, Smith JF. 2012. Phylogeny of a Neotropical clade in the Gesneriaceae: more tales of convergent evolution. *International Journal of Plant Sciences* **173:** 894–916.

Clark JL, Herendeen PS, Skog LE, Zimmer EA. 2006. Phylogenetic relationships and generic boundaries in the Episcieae (Gesneriaceae) inferred from nuclear, chloroplast, and morphological data. *Taxon* **55:** 313–336.

Clark JL, Neill DA, Weber A, Gruhn JA, Katan T. 2010. *Shuaria* (Gesneriaceae), an arborescent new genus from the Cordillera del Condor and Amazonian Ecuador. *Systematic Botany* **35:** 662–674.

Clark JR, Ree RH, Alfaro ME, King MG, Wagner WL, Roalson EH. 2008. A comparative study in ancestral range reconstruction methods: retracing the uncertain histories of insular lineages. *Systematic Biology* **57:** 693–707.

Clark JL, Zimmer EA. 2003. A preliminary phylogeny of Alloplectus (Gesneriaceae): implications for the evolution of flower resupination. *Systematic Botany* **28:** 365–375.

Conran JG, Christophel DC. 2004. A fossil Byblidaceae seed from Eocene South Australia. *International Journal of Plant Sciences* **165:** 691–694.

Cosacov A, Sersic AN, Sosa V, De-Nova JA, Nylinder S, Cocucci AA. 2009. New insights into the phylogenetic

relationships, character evolution, and phytogeographic patterns of *Calceolaria* (Calceolariaceae). *American Journal of Botany* **96:** 2240–2255.

Costa LP. 2003. The historical bridge between the Amazon and the Atlantic forest of Brazil: a study of molecular phylogeography with small mammals. *Journal of Biogeography* **30:** 71–86.

Cronk QCB, Kiehn M, Wagner WL, Smith JE. 2005. Evolution of *Cyrtandra* (Gesneriaceae) in the Pacific Ocean: the origin of a supertramp clade. *American Journal of Botany* **92:** 1017–1024.

Davidson DW. 1988. Ecological-studies of Neotropical ant gardens. *Ecology* **69:** 1138–1152.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4:** 699–710.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214.

Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **5:** 1–19.

Feuillet C. 2009. Checklist of the plants of the Guiana shield 1. An update to the angiosperms. *Journal of the Botanical Research Institute of Texas* **3:** 799–814.

Fiaschi P, Pirani JR. 2009. Review of plant biogeographic studies in Brazil. *Journal of Systematics and Evolution* **47:** 477–496.

Fischer E. 1997. Contribution to the flora of Central Africa VI: *Stemodiopsis* Engl. (Scrophulariaceae) in Central Africa (Zaire, Rwanda, Burundi) with remarks on an overlooked species of *Crepidorhopalon* E. Fischer. *Bulletin du Jardin Botanique National de Belgique* **66:** 73–79.

Fischer TC, Butzmann R. 2006. The infructescens of *Paulownia inopinata* Butzmann & Fischer – emendation of the species definition. *Documenta naturae* **155:** 1–7.

Fouquet A, Recoder R, Teixeira M, Cassimiro J, Amaro RC, Camacho A, Damasceno R, Carnaval AC, Moritz C, Rodrigues MT. 2012. Molecular phylogeny and morphometric analyses reveal deep divergence between Amazonia and Atlantic Forest species of *Dendrophryniscus*. *Molecular Phylogenetics and Evolution* **62:** 826–838.

Garzione CN, Hoke GD, Libarkin JC, Withers S, MacFadden B, Eiler J, Ghosh P, Mulch A. 2008. Rise of the Andes. *Science* **320:** 1304–1307.

Gentry AH. 1982. Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden* **69:** 557–593.

Givnish TJ, Barfuss MHJ, Van Ee B, Riina R, Schulte K, Horres R, Gonsiska PA, Jabaily RS, Crayn DM, Smith JAC, Winter K, Brown GK, Evans TM, Holst BK, Luther H, Till W, Zizka G, Berry PE, Sytsma KJ. 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. *American Journal of Botany* **98:** 872–895.

Hartl D. 1956. Morphologische Studien am Pistill der Scrophulariaceen. *Österreichische Botanishe Zeitschrift* **103:** 185–242.

Hilu KW, Borsch T, Muller K, Soltis DE, Soltis PS, Savolainen V, Chase MW, Powell MP, Alice LA, Evans R, Sauquet H, Neinhuis C, Slotta TAB, Rohwer JG, Campbell CS, Chatrou LW. 2003. Angiosperm phylogeny based on *matK* sequence information. *American Journal of Botany* **90:** 1758–1776.

Hiruma ST, Riccomini C, Modenesi-Gauttieri MC, Hackspacher PC, Neto JCH, Franco-Magalhaes AOB. 2010. Denudation history of the Bocaina Plateau, Serra do Mar, southeastern Brazil: relationships to Gondwana breakup and passive margin development. *Gondwana Research* **18:** 674–687.

Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler T, Sarkinen T, Antonelli A. 2010. Amazonia through time: andean uplift, climate change, landscape evolution, and biodiversity. *Science* **330:** 927–931.

Iturralde-Vinent MA, MacPhee RDE. 1999. Paleogeography of the Caribbean region: implications for Cenozoic biogeography. *Bulletin of the American Museum of Natural History* **238:** 1–95.

Janssens SB, Knox EB, Huysmans S, Smets EF, Merckx VSFT. 2009. Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: result of a global climate change. *Molecular Phylogenetics and Evolution* **52:** 806–824.

Jensen SR. 1996. Caffeoyl phenylethanoid glycosides in *Sanango racemosum* and in the Gesneriaceae. *Phytochemistry* **43:** 777–783.

Jensen SR. 2000. Chemical relationships of *Polypremum procumbens*, *Tetrachondra hamiltonii* and *Peltanthera floribunda*. *Biochemical Systematics and Ecology* **28:** 45–51.

Kleinfeldt SE. 1978. Ant-gardens interaction of *Codonanthe crassifolia* (Gesneriaceae) and *Crematogaster longispina* (Formicidae). *Ecology* **59:** 449–456.

Knapp M, Stockler K, Havell D, Delsuc F, Sebastiani F, Lockhart PJ. 2005. Relaxed molecular clock provides evidence for long-distance dispersal of *Nothofagus* (southern beech). *PLoS Biology* **3:** 38–43.

Kvist LP, Skog LE, Amaya-Márquez M, Salinas I. 2005. Las Gesneriáceas de Perú. *Arnaldoa* **12:** 16–40.

Ledru MP, Rousseau DD, Cruz FW, Riccomini C, Karmann I, Martin L. 2005. Paleoclimate changes during the last 100,000 yr from a record in the Brazilian Atlantic rainforest region and interhemispheric comparison. *Quaternary Research* **64:** 444–450.

Lewis DQ. 2000. A revision of the New World species of *Lindernia* (Scrophulariaceae). *Castanea* **65:** 93–122.

Linder HP. 2008. Plant species radiations: where, when, why? *Philosophical Transactions of the Royal Society B* **363:** 3097–3105.

Maddison DR, Maddison WP. 2003. *MacClade, version 4.06*. Sunderland, MA: Sinauer Associates.

Manchester SR, Chen ZD, Lu AM, Uemura K. 2009. Eastern Asian endemic seed plant genera and their paleogeographic history throughout the Northern Hemisphere. *Journal of Systematics and Evolution* **47:** 1–42.

Marten-Rodriguez S, Almarales-Castro A, Fenster CB. 2009. Evaluation of pollination syndromes in Antillean Gesneriaceae: evidence for bat, hummingbird and generalized flowers. *Journal of Ecology* **97:** 348–359.

Marten-Rodriguez S, Fenster CB, Agnarsson I, Skog LE, Zimmer EA. 2010. Evolutionary breakdown of pollination specialization in a Caribbean plant radiation. *New Phytologist* **188:** 403–417.

Mayer V, Moller M, Perret M, Weber A. 2003. Phylogenetic position and generic differentiation of Epithemateae (Gesneriaceae) inferred from plastid DNA sequence data. *American Journal of Botany* **90:** 321–329.

Meudt HM, Simpson BB. 2006. The biogeography of the austral, subalpine genus Ourisia (Plantaginaceae) based on molecular phylogenetic evidence: South American origin and dispersal to New Zealand and Tasmania. *Biological Journal of the Linnean Society* **87:** 479–513.

Möller M, Pfosser M, Jang CG, Mayer V, Clark A, Hollingsworth ML, Barfuss MHJ, Wang YZ, Kiehn M, Weber A. 2009. A preliminary phylogeny of the 'didymocarpoid Gesneriaceae' based on three molecular data sets: incongruence with available tribal classifications. *American Journal of Botany* **96:** 989–1010.

Moore BR, Donoghue MJ. 2007. Correlates of diversification in the plant clade Dipsacales: geographic movement and evolutionary innovations. *American Naturalist* **170:** S28–S55.

Mora A, Baby P, Roddaz M, Parra M, Brusset S, Hermoza W, Espurt N. 2010. Tectonic history of the Andes and sub-Andean zones: implications for the development of the Amazon drainage basin. In: Hoorn C, Wesselingh FP, eds. *Amazonia, landscape and species evolution: a look into the past*. Oxford: Wiley, 38–60.

Morley RJ. 2003. Interplate dispersal paths for megathermal angiosperms. *Perspectives in Plant Ecology Evolution and Systematics* **6:** 5–20.

Müller K, Borsch T, Legendre L, Porembski S, Theisen I, Barthlott W. 2004. Evolution of carnivory in Lentibulariaceae and the Lamiales. *Plant Biology* **6:** 477–490.

Near TJ, Sanderson MJ. 2004. Assessing the quality of molecular divergence time estimates by fossil calibrations and fossil-based model selection. *Philosophical Transactions of the Royal Society B* **359:** 1477–1483.

Nylinder S, Swenson U, Persson C, Janssens SB, Oxelman B. 2012. A dated species-tree approach to the trans-Pacific disjunction of the genus *Jovellana* (Calceolariaceae, Lamiales). *Taxon* **61:** 381–391.

Oliveira AT, Fontes MAL. 2000. Patterns of floristic differentiation among Atlantic forests in southeastern Brazil and the influence of climate. *Biotropica* **32:** 793–810.

Oxelman B, Backlund M, Bremer B. 1999. Relationships of the Buddlejaceae *s. l.* Investigated using parsimony jackknife and branch support analysis of chloroplast *ndhF* and *rbcL* sequence data. *Systematic Botany* **24:** 164–182.

Oxelman B, Liden M, Berglund D. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution* **206:** 393–410.

Pennington RT, Lavin M, Prado DE, Pendry CA, Pell SK, Butterworth CA. 2004. Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. *Philosophical Transactions of the Royal Society B-Biological Sciences* **359:** 515–537.

Pennington RT, Lewis GP, Ratter JA. 2006. An overview of the plant diversity, biogeography and conservation of Neotropical savannas and seasonally dry forests. In: Pennington RT, Lewis GP, Ratter JA, eds. *Neotropical savannas and seasonally dry forests: plant diversity, biogeography and conservation*. Boca Raton, FL: CRC Press, 1–29.

Perret M, Chautems A, Spichiger R. 2006. Dispersal-vicariance analyses in the tribe Sinningieae (Gesneriaceae): a clue to understanding biogeographical history of the Brazilian Atlantic forest. *Annals of the Missouri Botanical Garden* **93:** 340–358.

Perret M, Chautems A, Spichiger R, Barraclough TG, Savolainen V. 2007. The geographical pattern of speciation and floral diversification in the Neotropics: the tribe Sinningieae (Gesneriaceae) as a case study. *Evolution* **61:** 1641–1660.

Perret M, Chautems A, Spichiger R, Kite G, Savolainen V. 2003. Systematics and evolution of tribe Sinningieae (Gesneriaceae): evidence from phylogenetic analyses of six plastid DNA regions and nuclear ncpGS. *American Journal of Botany* **90:** 445–460.

Pigg KB, Wehr WC. 2002. Tertiary flowers, fruits, and seeds of Washington State and adjacent areas – Part III. *Washington Geology* **30:** 3–16.

Pirie MD, Chatrou LW, Mols JB, Erkens RHJ, Oosterhof J. 2006. 'Andean-centred' genera in the short-branch clade of Annonaceae: testing biogeographical hypotheses using phylogeny reconstruction and molecular dating. *Journal of Biogeography* **33:** 31–46.

Rahmanzadeh R, Muller K, Fischer E, Bartels D, Borsch T. 2005. The Linderniaceae and Gratiolaceae are further lineages distinct from the Scrophulariaceae (Lamiales). *Plant Biology* **7:** 67–78.

Raven PH, Axelrod DI. 1974. Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden* **61:** 539–673.

Ree RH, Moore BR, Webb CO, Donoghue MJ. 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution* **59:** 2299–2311.

Ree RH, Smith SA. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* **57:** 4–14.

Renner SS. 2005. Relaxed molecular clocks for dating historical plant dispersal events. *Trends in Plant Science* **10:** 550–558.

Riccomini C, Peloggia AUG, Saloni JCL, Kohnke MW, Figueira RM. 1989. Neotectonic activity in the Serra do Mar rift system (southeastern Brazil). *Journal of South American Earth Sciences* **2:** 191–197.

Roalson EH, Boggan JK, Skog LE. 2005a. Reorganization of tribal and generic boundaries in the Gloxinieae (Gesneriacea: Gesnerioideae) and the description of a new tribe in the Gesnerioideae, Sphaerorrhizeae. *Selbyana* **25:** 225–238.

Roalson EH, Boggan JK, Skog LE, Zimmer EA. 2005b. Untangling Gloxinieae (Gesneriaceae). I. Phylogenetic patterns and generic boundaries inferred from nuclear, chloroplast, and morphological cladistic datasets. *Taxon* **54:** 389–410.

Roalson EH, Skog LE, Zimmer EA. 2008. Untangling Gloxinieae (Gesneriaceae). II. Reconstructing biogeographic patterns and estimating divergence times among New World continental and island lineages. *Systematic Botany* **33:** 159–175.

Salinas F, Armesto JJ. 2012. Regeneration niche of three epiphytic species of Gesneriaceae from Chilean rainforests: implications for the evolution of growth habits in Coronanthereae. *Botanical Journal of the Linnean Society* **170:** 79–92.

Salinas MF, Arroyo MTK, Armesto JJ. 2010. Epiphytic growth habits of Chilean Gesneriaceae and the evolution of epiphytes within the tribe Coronanthereae. *Annals of the Missouri Botanical Garden* **97:** 117–127.

Sanderson MJ. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution* **14:** 1218–1231.

Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* **19:** 101–109.

Sang T, Crawford DJ, Stuessy TF. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* **84:** 1120–1136.

Sanmartín I, Ronquist F. 2004. Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology* **53:** 216–243.

Sanmartin-Gajardo I, Sazima M. 2004. Non-euglossine bees also function as pollinators of *Sinningia* species (Gesneriaceae) in southeastern Brazil. *Plant Biology* **6:** 506–512.

Sanmartin-Gajardo I, Sazima M. 2005. Chiropterophily in Sinningieae (Gesneriaceae): *Sinningia brasiliensis* and *Paliavana prasinata* are bat-pollinated, but *P. sericiflora* is not. Not yet? *Annals of Botany* **95:** 1097–1103.

Sanmartin-Gajardo I, Vianna JRS. 2010. Pollination of *Nematanthus brasiliensis*: an epiphytic Gesneriaceae endemic to the southeastern Atlantic forests of Brazil. *Selbyana* **30:** 216–220.

Santos AMM, Cavalcanti DR, da Silva JMC, Tabarelli M. 2007. Biogeographical relationships among tropical forests in north-eastern Brazil. *Journal of Biogeography* **34:** 437–446.

Santos JC, Coloma LA, Summers K, Caldwell JP, Ree R, Cannatella DC. 2009. Amazonian amphibian diversity is primarily derived from Late Miocene Andean lineages. *PLoS Biology* **7:** 448–461.

Schäferhoff B, Fleischmann A, Fischer E, Albach DC, Borsch T, Heubl G, Muller KF. 2010. Towards resolving Lamiales relationships: insights from rapidly evolving chloroplast sequences. *BMC Evolutionary Biology* **10:** 352.

Silvestro D, Schnitzler J, Zizka G. 2011. A Bayesian framework to estimate diversification rates and their variation through time and space. *BMC Evolutionary Biology* **11:** 311.

Simon MF, Grether R, de Queiroz LP, Skema C, Pennington RT, Hughes CE. 2009. Recent assembly of the cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proceedings of the National Academy of Sciences of the United States of America* **106:** 20359–20364.

Skog LE. 1976. A study of the tribe Gesnerieae with a revision of *Gesneria* (Gesneriaceae: Gesnerioideae). *Smithsonian Contributions to Botany* **29:** 1–182.

Skog LE. 1979. Flora of Panama, Family 175, Gesneriaceae. *Annals of the Missouri Botanical Garden* **65:** 783–996.

Skog LE. 1999. Gesneriaceae. In: Jørgenson PM, León-Yánez S, eds. *Catalogue of the vascular plants of Ecuador*. St. Louis, MO: Missouri Botanical Garden Press, 492–507.

Skog LE, Boggan JK. 2006. A new classification of the Western Hemisphere Gesneriaceae. *Gesneriads* **56:** 12–17.

Skog LE, Boggan JK. 2011. *World checklist of Gesneriaceae*. Washington, DC: Department of Botany, Smithsonian Institution. Available at: http://persoon.si.edu/gesneriaceae/checklist

Smith JF. 1996. Tribal relationships within Gesneriaceae: a cladistic analysis of morphological data. *Systematic Botany* **21:** 497–513.

Smith JF. 2001. High species diversity in fleshy-fruited tropical understory plants. *American Naturalist* **157:** 646–653.

Smith JF, Brown KD, Carroll CL, Denton DS. 1997. Familial placement of *Cyrtandromoea*, *Titanotrichum* and *Sanango*, three problematic genera of the Lamiales. *Taxon* **46:** 65–74.

Sytsma KJ, Litt A, Zjhra ML, Pires JC, Nepokroeff M, Conti E, Walker J, Wilson PG. 2004. Clades, clocks, and continents: historical and biogeographical analysis of Myrtaceae, Vochysiaceae, and relatives in the Southern Hemisphere. *International Journal of Plant Sciences* **165:** S85–S105.

Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of 3 noncoding regions of chloroplast DNA. *Plant Molecular Biology* **17:** 1105–1109.

Taylor DW. 1991. Paleobiogeographic relationships of Andean angiosperms of Cretaceous to Pliocene age. *Palaeogeography Palaeoclimatology Palaeoecology* **88:** 69–84.

Thorn VC, DeConto R. 2006. Antarctic climate at the Eocene/Oligocene boundary – climate model sensitivity to high latitude vegetation type and comparisons with the palaeobotanical record. *Palaeogeography Palaeoclimatology Palaeoecology* **231:** 134–157.

Trenel P, Gustafsson MHG, Baker WJ, Asmussen-Lange CB, Dransfield J, Borchsenius F. 2007. Mid-Tertiary dispersal, not Gondwanan vicariance explains distribution patterns in the wax palm subfamily (Ceroxyloideae: Arecaceae). *Molecular Phylogenetics and Evolution* **45:** 272–288.

Wang CN, Cronk QCB. 2003. Meristem fate and bulbil formation in *Titanotrichum* (Gesneriaceae). *American Journal of Botany* **90:** 1696–1707.

Wang CN, Möller M, Cronk QCB. 2004a. Phylogenetic position of *Titanotrichum oldhamii* (Gesneriaceae) inferred from four different gene regions. *Systematic Botany* **29:** 407–418.

Wang CN, Möller M, Cronk QCB. 2004b. Population genetic structure of *Titanotrichum oldhamii* (Gesneriaceae), a subtropical bulbiliferous plant with mixed sexual and asexual reproduction. *Annals of Botany* **93:** 201–209.

Wang XF, Auler AS, Edwards RL, Cheng H, Cristalli PS, Smart PL, Richards DA, Shen CC. 2004. Wet periods in northeastern Brazil over the past 210 kyr linked to distant climate anomalies. *Nature* **432:** 740–743.

Weber A. 1971. Zur Morphologie des Gynoeceums der Gesneriaceen. *Österreichische Botanishe Zeitschrift* **119:** 234–305.

Weber A. 2004. Gesneriaceae. In: Kubitzki K, ed. *The families and genera of vascular plants*, Vol. 7. Berlin: Springer, 63–158.

Weber A, Skog LE. 2007. (onwards). *The genera of Gesneriaceae*. Basic information with illustration of selected species. Ed. 2. Available at: http://www.genera-gesneriaceae.at/

Wesley CW, Hopkins DQ. 1994. The Eocene orchards and gardens of Republic, Washington. *Washington Geology* **22:** 27–34.

Wiehler H. 1976. Report on classification of *Achimenes*, *Eucodonia*, *Gloxinia*, *Goyazia*, and *Anetanthus* (Gesneriaceae). *Selbyana* **1:** 374–404.

Wiehler H. 1978. The genera *Episcia*, *Alsobia*, *Nautilocalyx*, and *Paradrymonia* (Gesneriaceae). *Selbyana* **5:** 11–60.

Wiehler H. 1983. A synopsis of the Neotropical Gesneriaceae. *Selbyana* **6:** 1–219.

Wiehler H. 1994. A reexamination of *Sanango racemosum*. 4. Its new systematic position in Gesneriaceae. *Taxon* **43:** 625–632.

Woo VL, Funke MM, Smith JF, Lockhart PJ, Garnock-Jones PJ. 2011. New World origins of Southwest Pacific Gesneriaceae: multiple movements across and within the South Pacific. *International Journal of Plant Sciences* **172:** 434–457.

Zimmer EA, Roalson EH, Skog LE, Boggan JK, Idnurm A. 2002. Phylogenetic relationships in the Gesnerioideae (Gesneriaceae) based on nrDNA ITS and cpDNA *trnL-F* and *trnE-T* spacer region sequences. *American Journal of Botany* **89:** 296–311.

SUPPORTING INFORMATION

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

Figure S1. Chronogram of Lamiales with 95% highest posterior density bars, based on BEAST analyses using four fossils and two secondary calibration points (letters A–F; see Table 1). Bayesian posterior probabilities are indicated above branches when higher than 0.5. The portion of the tree corresponding to Gesneriaceae is shown in Figure 2.

Figure S2. Chronogram showing all the accessions analysed with 95% highest posterior density bars, based on BEAST analyses using four fossils and two secondary calibration points listed in Table 1. Bayesian posterior probabilities are indicated above branches when higher than 0.5. Geological time scale is shown at the bottom. The basal portion of the tree, including all other taxa of Lamiales, is available in Figure S1.

Figure S3. Palaeogeographical model used in the Lagrange analysis, with two time slices reflecting the probability of area connectivity through time. The area codes correspond to A, Tropical Andes (> 1000 m); B, Brazilian Atlantic forest; C, Central America; E, Cerrado; T, Temperate Andes; Z, Amazonian rainforest including lowland Amazonia, Sub-Andes, Guiana shield and northern Venezuela-Colombia; and W, West Indies. Taxa ranging outside America were assigned to either (O) Old World (Africa, Europe and tropical Asia) or (P) south-west Pacific (Australia, New Caledonia, New Zealand and Lord Howe Island).

Figure S4. Cross-validation procedure for the fossil calibrations used in this study. Letters refer to the calibration points defined in Table 1. The orange density plots represent the prior distribution used and the blue density plots give the posterior density obtained when the fossil was not used in the analyses. The large overlap between the prior and posterior densities observed for each calibration point indicates that the fossil calibrations are congruent.

Table S1. Taxa, specimens and GenBank accession number for sequences of Gesneriaceae used in the present study.

Table S2. Range of each terminal taxon coded as presence–absence. The area codes are indicated in the legend to Figure S3.

Text S1. Python script used for the Lagrange analysis shown in Figure 3.