

Untangling Gloxinieae (Gesneriaceae). II. Reconstructing Biogeographic Patterns and Estimating Divergence Times Among New World Continental and Island Lineages

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Abstract—Gesneriaceae tribe Gloxinieae is a diverse clade of approximately 19 genera and 215 species. As with many tropical lineages, patterns and timing of diversification are poorly understood. This is a particular difficulty in groups such as the Gesneriaceae that have no fossil record. Here we explore maximum likelihood and Bayesian inference of phylogenetic relationships in the tribe based on nuclear, chloroplast, and morphological data sets, use Fitch parsimony optimization (FPO) and dispersal vicariance (DIVA) analyses to explore biogeographic patterns in the Gesnerioideae, and use penalized likelihood calibrated by geological events in the Caribbean and South America to explore timing of movement of lineages among Caribbean, Central American, and South American land masses and islands. Likelihood and Bayesian analyses increase support of previous hypotheses of relationships using parsimony and provide additional resolution in some parts of the phylogeny. FPO and DIVA analyses suggest that the most likely scenario for movement among Central American, Caribbean, and South American areas was either an early dispersal to Central America and the Caribbean prior to diversification of the Gloxinieae clade with subsequent back dispersal to South America, or the ancestor of the Gloxinieae had a broad distribution across Central America and Andean/western South America. Estimations of the timing of movement of these lineages among these land masses suggests that the Greater Antilles/Aves Ridge landbridge likely played a role in dispersal events and that the Gloxinieae/Gesneriaceae lineage likely arrived in the Central America/Caribbean zone at least 26 million years ago.

Keywords—Bayesian inference, biogeography, Caribbean, divergence dating, Gesneriaceae, Gloxinieae.

Gesneriaceae tribe Gloxinieae is a morphologically diverse clade currently considered to include approximately 19 genera and 215 species (Roalson et al. 2005a). This tribe forms a well-defined and exclusively New World clade of subfamily Gesnerioideae (Zimmer et al. 2002; Roalson et al. 2005a). Historically, this group has been extremely problematic to classify due to a complex pattern of diversification and convergence in floral and vegetative forms. This has led to numerous reorganizations of generic boundaries in the tribe (e.g. Wiehler 1983) based on traditional systematic lines of evidence. Only recently have reasonably consistent and resolved phylogenetic patterns of this tribe begun to emerge (Zimmer et al. 2002; Roalson et al. 2003, 2005a). While this tribe has been included in several molecular phylogenetic studies over the past 10 yr (Smith 1996; Smith and Carroll 1997; Smith et al. 1997a, b; Smith and Atkinson 1998; Smith 2000c, 2001; Smith et al. 2004a, b), these studies did not provide consistent nor well-supported branching patterns in the Gloxinieae (for a more complete discussion see Zimmer et al. 2002).

While considerable attention has been paid to morphology, classification, and phylogeny in the Gesneriaceae, little attention has been paid to the historical biogeography of the family, or of any major clades within the family. To date, only Old World members of the family have been discussed in this context (Möller and Cronk 1997; Burt 1998; Denduangboripant and Cronk 2000; Atkins et al. 2001; Denduangboripant et al. 2001; Mendum et al. 2001; Möller and Cronk 2001). As with many tropical groups, neotropical gesneriads are very poorly understood with regards to biogeographic history, with effectively no studies exploring potential explanations of distribution patterns in the family in the New World.

Part of the difficulty of determining biogeographic patterns in the Gesneriaceae is the lack of any fossil pollen or macrofossils. Many other lineages of the Lamiales also have

poor fossil records, and the earliest fossil pollen records from families including the Lamiaceae, Acanthaceae, Orobanchaceae, and Plantaginaceae do not show up until the lower Miocene (~15–25 million years ago [MYA]) at the earliest (Muller 1981; Wiehler 1983). There seems to be a very different inference of the timing of diversification of Lamiales lineages based upon these pollen records as opposed to those inferences derived from our current evidence of phylogenetic relationships. The Gesneriaceae and Oleaceae appear to be two of the earliest diverging lineages of the Lamiales (Oxelman et al. 1999; Olmstead et al. 2000), but the pollen record for the Gesneriaceae appears to be completely absent and Oleaceae pollen has been dated as early as the Oligocene (~25–35 MYA; Muller 1981). The Bignoniaceae, which does not diverge from its sister taxa (likely some portion of the Verbenaceae, Scrophulariaceae, or Buddlejaceae; Oxelman et al. 1999; Olmstead et al. 2000) until substantially later than the origin of the Oleaceae or Gesneriaceae lineages has pollen dated from the middle Eocene, 15–20 MYA prior to the earliest record of the Oleaceae. Similarly, macrofossils of *Acanthus* (Acanthaceae) have been found as early as the Eocene, despite the more recent divergence of the Acanthaceae from other Lamiales lineages (Raven and Axelrod 1974). These findings suggest that either the pollen and/or macrofossils from the Lamiales need to be reassessed with regards to current phylogenetic/classification hypotheses, or the fossil record is exceedingly misrepresentative of the true antiquity of several of these lineages, and particularly the Gesneriaceae.

Comparison of Gesneriaceae diversity and biogeography in relation to plate tectonics led Raven and Axelrod (1974) to suggest that, given the distribution of subfamily Gesnerioideae in South America and the distribution of the Cyrtandroideae in Africa and Australasia, the family likely dates to a time when South America and Africa were in close physical

proximity, with the divergence of these lineages associated with the separating of these continents (continental vicariance). This would place the origins of the family prior to the Mesozoic/Cenozoic boundary at 65 MYA, as these two continents were reasonably separated by this time (McLoughlin 2001). Alternatively, the origin of the subfamilies may have postdated the division of South America and Africa and their distribution may be the result of more recent long-distance dispersal between these regions.

Large-scale estimation of nodal ages of major angiosperm clades has suggested a wide range of potential dates for the origin of the Lamiales clade, including as early as 71–74 MYA (Wikström et al. 2001) or as late as 44.25 MYA (Magallón and Sanderson 2001). Bremer et al. (2004) estimated a date for the age of the crown group Gesneriaceae at 71 MYA based on analyses of divergence dates across the Asterids. It should be noted, however, that current methods of inferring ages at phylogenetic nodes can be substantially influenced by phylogenetic uncertainty, substantial noise, and lineage effects, creating exceptionally large confidence intervals around age estimates (Sanderson and Doyle 2001).

Similar difficulty lies in understanding the biogeographic patterns within the Gesnerioideae lineage and the patterns of diversity found in different regions of South America, Central America, and the Caribbean. Some of this comes from incomplete understanding of the diversity and distributions of these lineages, but is also associated with the previous dependence on “centers of diversity” to explain biogeographic patterns (Gentry 1982; Kvist and Skog 1992, 1996; Skog and Kvist 2000; Weigend and Förther 2002; Perret et al. 2003). While there are potential problems with the application of the “centers of diversity” method to understanding biogeography, it should be noted that these authors are among very few Gesneriaceae researchers who have even made an attempt to address biogeographic patterns, and the lack of discussion of biogeographic issues in major works on Gesneriaceae (Wiehler 1976, 1983; Smith and Carroll 1997; Smith and Atkinson 1998; Smith 2000a, 2000b, 2001) underscores this problem.

It is clear that the largest diversity of New World Gesneriaceae is found in northwestern South American midelevation rainforests and cloud forests (Gentry 1982; Kvist and Skog 1992, 1996; Skog and Kvist 2000), although some lineages are quite diverse in other regions (e.g. *Sinningia* in southeastern Brazil, Perret et al. 2003; *Achimenes* in Mexico, Ramírez Roa 1987; *Gesneria* in the Greater Antilles, Skog 1976). The traditional view that movement of lineages into other areas of the Neotropics was primarily unidirectional from this center of diversity is likely overly simplistic. However, alternative hypotheses of lineage movement have not been presented and the Gesneriaceae literature lacks any reference to biogeographic patterns outside of mapping locations of small groups under study (e.g. Kvist and Skog 1996).

The plate tectonic movements and origins of current landforms in northern South America, Central America, and the Caribbean Islands are exceedingly complex (Raven and Axelrod 1974; Coney 1982; Burnham and Graham 1999; Iturralde-Vinent and MacPhee 1999). While the timing of the Panamanian land bridge between Central America and Colombia is solidly placed between 3.0 and 3.5 MYA (Stehli and Webb 1985; Knowlton et al. 1993), there is some question about the presence and persistence of other land masses that might have facilitated movement of angiosperms among the

larger land masses. Particularly of interest are the island chains creating potential links between northwestern South America and Central America (Raven and Axelrod 1974; Iturralde-Vinent and MacPhee 1999), and the Lesser Antillean island chains and Greater Antilles/Aves Ridge landbridge (GAARLANDIA) thought to connect northeastern South America to the Greater Antilles and into close proximity to the North American land mass as early as 35 MYA (Iturralde-Vinent and MacPhee 1999). The potential role of these bridges and island chains in movement of the Gesneriaceae to the Caribbean and North and Central America is unclear, and, as discussed by Raven and Axelrod (1974), different lineages of Gesneriaceae likely arrived in these areas at different times, and therefore likely were differentially affected by the various possible paths. Some lineages, such as the Gesneriaceae tribe, are quite prominent in the West Indies supporting “the notion of a Paleogene arrival” (Raven and Axelrod 1974), while *Columnnea* with 11 endemic species on Jamaica might provide evidence for their arrival “early in Neogene time” (Raven and Axelrod 1974). These estimated arrival times were loosely based on the numbers of island endemics in these genera. Colonization of Caribbean islands prior to Late Eocene (~35 MYA) is not generally considered plausible as there were no permanent land masses prior to this epoch (Iturralde-Vinent and MacPhee 1999).

In order to start to understand biogeographic patterns and potential timing and mechanisms of dispersal, geographic distributions of lineages need to be placed in a phylogenetic context. Here we explore biogeographic patterns in the Gesneriaceae and particularly of tribe Gloxinieae, using phylogenetic hypotheses derived from maximum likelihood and Bayesian inference analyses of a variety of molecular and morphological data sets. The Gloxinieae are particularly appropriate for dating the movement among the New World land masses because of the presence of diverse lineages within this tribe largely restricted to one or a few of the geographic areas.

Likelihood and Bayesian phylogenetic hypotheses provide a framework for the exploration of historical biogeographic patterns with Fitch parsimony optimization and dispersal-vicariance analyses. Furthermore, the timing of biogeographic movements in the Gesnerioideae are addressed by dating nodes using plate tectonic and land bridge geologic time estimates and semiparametric rate smoothing by penalized likelihood methods. These results are discussed in the framework of previous biogeographic hypotheses and the potential for future dating of ancestral nodes in the Gloxinieae and Gesneriaceae as a whole is explored.

MATERIALS AND METHODS

Taxon Sampling, Gesnerioideae—Samples included in this study are those used in previous phylogenetic analysis of relationships in the Gesnerioideae (Zimmer et al. 2002; Roalson et al. 2005a). This includes 34 genera and 58 species in the Gesnerioideae and uses members of the Cyrtandroideae (*Aeschynanthus hildebrandii* and *Streptocarpus primulifolius*) as outgroups (Zimmer et al. 2002). The three data sets include nrDNA internal transcribed spacer (ITS) sequences, cpDNA *trnL* intron and *trnL-F* intergenic spacer (*trnL-F*) sequences, and cpDNA *trnE-T* intergenic spacer sequences (*trnE-T*). Details of these datasets have been previously enumerated, and will not be repeated here. All samples used in this study are vouchered or presented in Zimmer et al. (2002) and Roalson et al. (2005a) and listed in Appendix 1. Nomenclature follows Roalson et al. (2005b). The data matrix and maximum likelihood tree have been deposited in TreeBASE (study number S1794).

Taxon Sampling, Gloxinieae—Samples included in this study are those used in previous phylogenetic analysis of relationships in the Gloxinieae (Roalson et al. 2005a) and Gesnerioideae (Zimmer et al. 2002). Gloxinieae analyses include 17 genera and 54 ingroup species with members of the Gesnerieae (six species in four genera) as outgroups (Roalson et al. 2005a). The three data sets include nrDNA internal transcribed spacer (ITS) sequences, cpDNA *trnL* intron and *trnL-F* intergenic spacer (*trnL-F*) sequences, and a morphological dataset. Details of these datasets have been previously enumerated, and will not be repeated here. All samples used in this study are vouchers or presented in Zimmer et al. (2002) and Roalson et al. (2005a) and listed in Appendix 1. Nomenclature changes suggested in Roalson et al. (2005b) are followed here and differ from the generic circumscriptions used in Zimmer et al. (2002) and Roalson et al. (2005a). The data matrix and maximum likelihood tree have been deposited in TreeBASE (S1794).

Maximum Likelihood Phylogeny, Gesnerioideae—Maximum likelihood (ML) analysis of the combined ITS/*trnL-F*/*trnE-T* dataset was performed using PAUP* 4.0b10 (Swofford 2001). Heuristic searches were employed (TBR branch swapping). ML analyses employed the general time reversible (GTR; Rodríguez et al. 1990) model with proportion of invariant sites (I) and gamma shape (G) parameters and empirical base frequencies (six substitution types: A/C, 0.8489; A/G, 1.5790; A/T, 0.4177; C/G, 0.8349; C/T, 3.1447; G/T, 1.0000; I = 0.2954; G = 0.4704; A, 0.3151; C, 0.1904; G, 0.1931; T, 0.3014). This model was chosen based on the results of analysis using DT_ModSel (Minin et al. 2003). DT_ModSel examines the fit of various substitution models to the data set using the Bayesian information criterion and additionally incorporates relative branch-length error estimates in a decision theory framework (Minin et al. 2003).

Maximum Likelihood Phylogeny and Tests of Alternative Topologies, Gloxinieae—Maximum likelihood (ML) analysis of the combined ITS/*trnL-F* dataset was performed using PAUP* 4.0b10 (Swofford 2001). Heuristic searches were employed (TBR branch swapping). ML analyses employed the Tamura and Nei (TrN; Tamura and Nei 1993) model with proportion of invariant sites (I) and gamma shape (G) parameters and empirical base frequencies (six substitution types: A/C, 1.0000; A/G, 2.5060; A/T, 1.0000; C/G, 1.0000; C/T, 4.3089; G/T, 1.0000; I = 0.4964; G = 0.6625; A, 0.3018; C, 0.1973; G, 0.2018; T, 0.2991). This model was chosen based on the results of analysis using DT_ModSel (see description above; Minin et al. 2003). One alternative topology was tested using the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 1999) in a maximum likelihood framework employing constraint options implemented in PAUP*: the topology forced monophyly of a clade containing all Central American genera (*Achimenes*, *Eucoadonia*, *Moussonina*, *Niphaea*, *Smithiantha*, and *Solenophora*). The SH analysis was run with 10000 RELL (Resampling of Estimated Log Likelihoods) bootstraps (one-tailed).

Bayesian Inference Analysis of Three Datasets, Gloxinieae—Bayesian inference analyses were performed using MrBayes v.3.0 (Huelsenbeck and Ronquist 2001). Three partitions were set to correspond with the ITS, *trnL-F*, and morphological datasets. The parameters for each dataset were allowed to vary independently (“unlinked”). Priors for the two molecular datasets included a model with six substitution types, rates following a gamma distribution (four categories), and allowing a proportion of invariant sites in the ITS model. These models were chosen based on the results of analyses using DT_ModSel (see description above; Minin et al. 2003). Priors for the morphological dataset were set at the default condition of all character changes equally probable and equally weighted. One hundred million generations were run with four Markov Chain Monte Carlo (MCMC) chains, and a tree was saved every 100 generations. The trees from the MrBayes analysis were loaded into PAUP*4.0b10 (Swofford 2001), discarding the trees sampled during the “burnin” of the chain (Huelsenbeck and Ronquist 2001; the first 20,000,000 generations or the first 200,001 trees) to only include trees after stationarity was reached. Examination of likelihood plots suggest that stationarity was reached within the designated burnin (data not shown). Additionally, multiple independent runs (five) starting from different random trees were conducted to determine if convergence and mixing had occurred. A majority rule consensus tree was made, showing nodes with a posterior probability of 50% or more. Majority rule consensus trees of the trees sampled in Bayesian inference analyses yielded probabilities that the clades are monophyletic (Lewis 2001). Additionally, five trees were randomly chosen (chosen using a random number generator at <http://random.org>) from the posterior probability distribution of trees for use in the character and biogeography reconstructions described below. These five trees represent different topologies from the posterior probability distribution of trees and therefore represent plausible topologies, although some nodes

on some trees might have a lower probability than others. Since biogeographic hypotheses are being explored across several plausible topologies, hypotheses on the evolutionary dynamics in the group will be more robust since they are not reliant on a single topology being correct at all nodes.

Reconstruction of Biogeographic Patterns—The historical biogeographic patterns in the Gesnerioideae and Gloxinieae datasets were inferred with standard Fitch parsimony optimization (FPO; accelerated transformation; as implemented in Mesquite 1.12; Maddison and Maddison 2006) and dispersal-vicariance analysis (DIVA; Ronquist 1996, 1997). The FPO method assumes that geographic distributions are the result of dispersal events. Polymorphic nodes are therefore restricted to terminal nodes and ancestral states are calculated by minimizing the number of character state changes on the tree. For these analyses geographic distribution was coded as a single multistate character based on distributions as described in Appendices 2 and 3 and analyzed using Mesquite 1.12 (Maddison and Maddison 2006). DIVA analysis, alternatively, assumes that geographic distributions can be the result of dispersal, extinction, and vicariance events. Polymorphic states are not restricted to terminals and ancestral states are calculated by minimizing the number of dispersal events necessary to explain the distribution pattern. Geographic distributions were coded as separate binary characters for presence/absence of each species in each geographic region based on distributions as described in Appendices 2 and 3 and the analysis was performed using DIVA 1.1a (Ronquist 1996).

The four areas described for FPO and DIVA analyses of the Gloxinieae were the Caribbean, Central America, Andean/western South America, and southern Brazil. Gesnerioideae analyses also included Old World and Guianan geographic areas. DIVA optimizations were conducted with either an unrestricted maximum number of areas assigned to each node or with the maximum number of areas restricted to two. This follows the reasoning that species of the Gesnerioideae are generally not found in more than two of the geographic regions described here, and that forcing internal nodes to not include all geographic regions may tell us more about patterns of dispersal and vicariance than would suggesting that ancestors were extremely widespread in the New World. Both FPO and DIVA analyses were run on all five of the trees randomly chosen from the posterior probability distribution of trees resulting from the Bayesian analysis, however, the primary differences in ancestral state reconstructions revolve around the paraphyly of the Gloxinieae Central American clades in relation to the Gloxinieae South American clade or the monophyly of the Central American clade. Therefore, a representative tree of each type is presented here, but it should be noted that other slightly different topologies had identical reconstructions of the nodes of interest (data not shown).

Estimating Absolute Divergence Times of Gesnerioideae Clades—There are many methods now available for phylogenetic dating (for reviews see Benton and Ayala 2003; Bromham and Penny 2003). Here we used the r8s program (Sanderson 2004) implementing semiparametric rate smoothing by penalized likelihood and the truncated Newton algorithm (Sanderson 2002) which uses a likelihood approach with a roughness penalty that prevents too much variation in rates. The roughness penalty is specified by a smoothing parameter derived from cross-validation analyses. Cross validation analyses (not shown) resulted in a smoothing parameter of 160.

The topology and branch lengths from the Gesnerioideae ML analysis were used as the starting point for estimating absolute divergence dates. Due to the r8s restriction that all nodes be resolved, unresolved branches from the ML analysis were resolved with very short branches using the topology found in Fig. 3, based on previous analyses. Five different constraint sets were used to assess divergence times on the ML topology. These constraint sets set maximum or maximum and minimum ages at two or three tree nodes and are referred to as “56/52,” “56/32,” “56/8,” “56/52/8,” and “56/32/8” (Table 1). All analyses included a maximum age at node 56 of 71 MYBP based on estimates of the stem age of the Gesneriaceae (Bremer et al. 2004). Bremer et al. (2004) included *Peltanthera* within their circumscription of Gesneriaceae for this estimate, which is a circumscription that other Gesneriaceae researchers disagree with (Wang et al. 2004). However, this date is used as a maximum for the Gesneriaceae stem age in this study, and is therefore appropriately used here even if *Peltanthera* is not considered part of Gesneriaceae. Geological evidence suggests that GAARLANDIA and persistent Caribbean land masses did not predate 35 MYBP and that the GAARLANDIA land bridge had been sundered by 25 MYBP. In order to test the possible association of the Gesnerieae + Gloxinieae stem and crown nodes with this land bridge, 25 and 35 MYBP were placed as minimum and maximum ages on nodes 52

TABLE 1. Constraint sets for r8s analyses. All times are given in millions of years ago (MYA). Node numbers refer to Fig. 5.

Constraint	Node 56	Node 52	Node 32	Node 8
56/52	Max = 71	Max = 35 Min = 25	—	—
56/32	Max = 71	—	Max = 35 Min = 25	—
56/8	Max = 71	—	—	Max = 16
56/52/8	Max = 71	Max = 35	—	Max = 16
56/32/8	Max = 71	—	Max = 35	Max = 16

and 32. By approximately 16 MYBP, a southern Central America tectonic block narrowed the gap between the North American and South American land masses. In order to explore this land mass' role in facilitating migration of the Gloxinieae back to South America, node 8 was constrained with a maximum age of 16 MYBP. Each of these three constraints was analyzed with the family age constraint individually. Combined constraint of nodes 56, 52, and 8 (but with a maximum constraint only on node 52), and nodes 56, 32, and 8 (but with a maximum constraint only on node 32) were each analyzed. All land mass ages were taken from Iturralde-Vinent and MacPhee (1999).

Confidence intervals were calculated by creating 100 bootstrap data matrices of the Gesnerioideae gene matrix using the SEQBOOT program in Felsenstein's (2004) PHYLIP package. These replicate data sets were used to estimate branch lengths on the ML topology and these phylogenies were then analyzed in r8s to create a 95% confidence interval of the age at each node.

RESULTS

Maximum Likelihood Phylogeny of the Gesnerioideae—The maximum likelihood analysis of the Gesnerioideae data set resulted in a single most likely tree ($-\ln L = 17313.46185$; Fig. 1). ML bootstrap analysis resulted in similar support for branches as previously found with parsimony analyses (Zimmer et al. 2002). This phylogenetic hypothesis resulted in all seven tribes here included forming monophyletic groups and, within the Gloxinieae, the two Central American clades forming a grade leading to the South American Gloxinieae clade (Fig. 1).

Maximum Likelihood and Bayesian Phylogenies and Tests of Alternative Topologies in the Gloxinieae—The maximum likelihood analysis of the Gloxinieae data set resulted in a single most likely tree ($-\ln L = 8989.95793$; Fig. 2). Bayesian inference analyses resulted in plots of log-likelihood scores and all other parameters reaching stationarity prior to generation 20,000,000 in all independent analyses (plots not shown). The first 200,001 sample points were thus discarded as burn-in, leaving 800,000 samples for construction of a 50% majority rule consensus tree. Figure 2 illustrates the ML topology with 50% posterior probability distributions from one of the runs mapped onto the phylogeny (results from this run are used in all further results and discussions). All five independent analyses resulted in similar posterior probability distributions and therefore we expect that convergence and mixing is occurring (data not shown). Sixty-four percent of nodes have a posterior probability $\geq 95\%$. The ML and Bayesian topologies are congruent but with some small differences either at unsupported nodes, or among closely related species. Both of these analyses of the combined molecular dataset present the two Central American clades as a grade leading to the South American Gloxinieae clade (Fig. 2). However, when we test the alternative topology with the Central American clades forced to form a sister clade to the South American clade, we do not find a significant difference

in likelihood score, as measured by the SH test ($P = 0.4221$). This is not unexpected as only a marginal majority of trees from the Bayesian posterior probability distribution of trees supported this branch of the highest likelihood tree (PP = 52%; Fig. 1).

Reconstruction of Biogeographic Patterns—The FPO biogeographic reconstruction of subfamily Gesnerioideae resulted in two different topologies, depending on whether the *Achimenes/Solenophora* and *Eucodonia/Smithiantha/Moussonia/Niphaea* clades (referred to in the future as the "Central American clades") are sister to each other or form a grade leading to the rest of the tribe. When the Central American clades form a grade leading to the rest of the Gloxinieae, the ancestral node of the Gloxinieae (and most of the rest of the internal nodes of the phylogeny) is equivocal (Fig. 3A). When these two clades are considered sister to one another, the ancestor of the Gloxinieae tribe is considered to be of Andean/western South American origin (Fig. 3B). Similarly, the ancestor of the Gesnerieae/Gloxinieae clade, the ancestor of the Episcieae + Sinningieae + *Sphaerorrhiza sarmentiana* + Gesnerieae + Gloxinieae clade, and the ancestor of subfamily Gesnerioideae are all reconstructed as being of Andean/western South American origin (Fig. 3B). The ancestral nodes of the Episcieae, Sinningieae, and *Sphaerorrhiza sarmentiana* lineages are all equivocal, and can be reconstructed as Andean/western South American or southern Brazilian in origin.

The DIVA Gesnerioideae biogeographic reconstruction resulted in 12 inferred dispersal/extinction events regardless of topology where the maximum areas for each node was unconstrained (Fig. 3A, B). When the Central American clades form a grade leading to the rest of the Gloxinieae, the ancestor of the Gloxinieae is reconstructed as being of Central American origin (Fig. 3A). However, where these clades are sister to one another (Fig. 3B), the ancestor of the Gloxinieae tribe is reconstructed as widespread through Central America and Andean/western South America or restricted to Central America. When the maximum areas at each node were restricted to two, either 15 or 16 dispersal events were necessary to reconstruct biogeographic patterns (Fig. 3A, B). The reconstruction of the ancestral state of the Gloxinieae was restricted to either a Central American origin or widespread in Central America and Andean/western South America if the Central American clades form a grade (Fig. 3A), but the reconstruction of this node when they are inferred to be sister clades was a widespread Andean/western South American and Central American distribution (Fig. 3B).

Biogeographic analysis of the Gloxinieae with FPO resulted in two different reconstructions of biogeographic patterns, both of six steps (Fig. 4A, B). The only areas of ambiguity in the reconstruction is where the Central American clades form a grade is at the root (Fig. 4A), and, where the Central American clades form a clade, the only ambiguous reconstructions are at the ancestor of the Gloxinieae and the root (Fig. 4B). The only differences between the two FPO patterns are the reconstruction of the ancestral state for tribe Gloxinieae and the number of alternative reconstructions at the Gloxinieae ancestor and root nodes.

DIVA analysis of the Gloxinieae topologies resulted in two possible patterns. DIVA pattern 1 resulted in an optimum reconstruction of five dispersal events whether constrained to two ancestral areas or unconstrained (Fig. 4A). DIVA pattern 2 (Fig. 4B) resulted in an optimum reconstruction of four

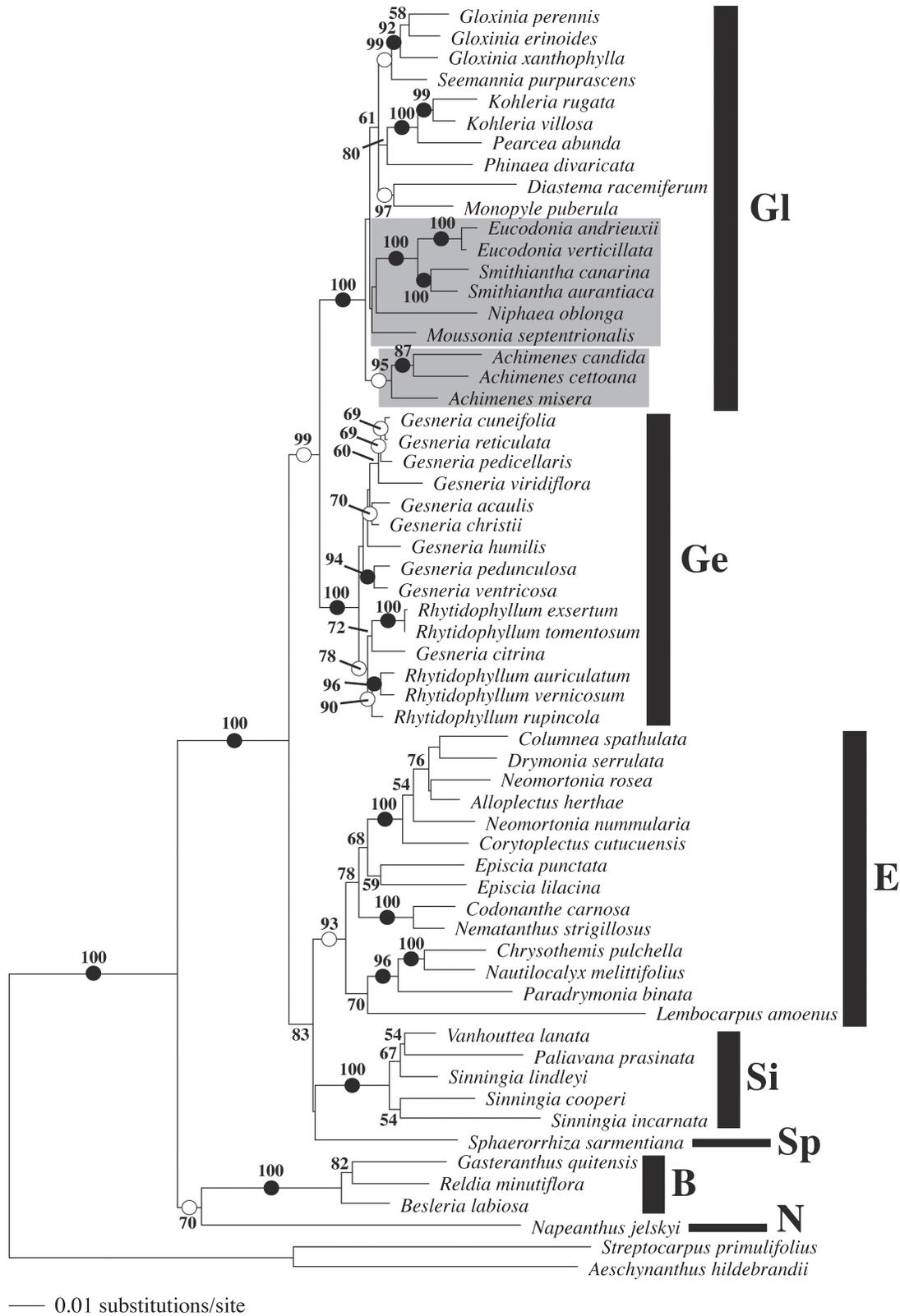


FIG. 1. Maximum likelihood phylogeny of Gesnerioideae relationships ($-\ln L = 17313.46185$). ML bootstrap values are noted above branches. Open circles on branches refer to branches with a maximum parsimony bootstrap percentage $\geq 70\%$ and closed circles on branches refer to branches with a maximum parsimony bootstrap percentage $\geq 95\%$ from previous analyses of the combined ITS/*trnL-F*/*trnE-T* combined dataset (Zimmer et al. 2002). Shaded boxes outline the two Central American Gloxinieae clades. Bars to the right of clades denote tribes abbreviated as follows: B = Beslerieae, E = Episceae, Ge = Gesnerieae, Gl = Gloxinieae, N = Napeantheae, Si = Sinningieae, and Sp = Sphaerorrhizeae.

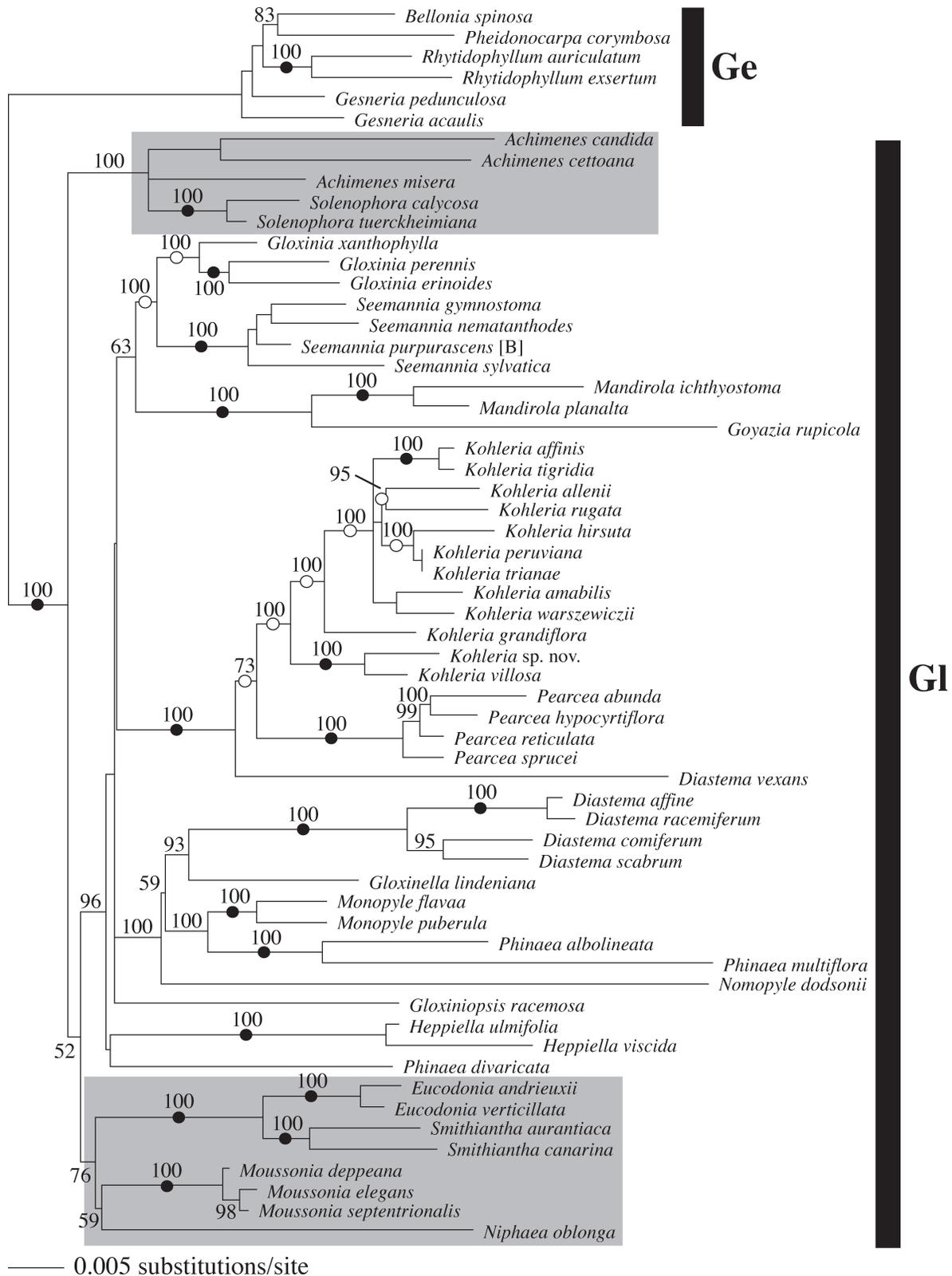


FIG. 2. Maximum likelihood phylogeny of Gloxinieae relationships ($-\ln L = 8989.95793$) based on the combined ITS/*trnL-F* dataset. Bayesian inference posterior probability values are noted above branches. Open circles on branches refer to branches with a maximum parsimony bootstrap percentage $\geq 70\%$ and closed circles on branches refer to branches with a maximum parsimony bootstrap percentage $\geq 95\%$ from previous analyses of the combined ITS/*trnL-F*/ morphology combined dataset (Roalson et al. 2005a). Shaded boxes outline the two Central American Gloxinieae clades. Bars to the right of clades denote tribes abbreviated as follows: Ge = Gesnerieae and Gl = Gloxinieae.

dispersal events when unconstrained, or five dispersals when constrained to two ancestral areas. The ancestral node of the Gloxinieae is alternatively resolved as either having a Central American distribution or widespread in Andean/western South America and Central America (Fig. 4A, B).

Estimating Absolute Divergence Times of Gesnerioideae Clades—The five constraint sets for estimating absolute divergence times in the Gesnerioideae all support very similar nodal ages (Table 2). Figure 5 diagrams the estimated nodal ages and confidence intervals under one of the five constraint sets (56/52/8). It should be noted, however, that the confidence intervals under all of the constraint sets overlap, suggesting that there is likely no significant difference among node age estimates under different constraints (Table 2). If the maximal age of the subfamily is less than 71 MYBP, as suggested by Bremer et al. (2004), the constraint of nodes associated with movements between South America, Central America, and the Caribbean to the estimated timing of land bridge or narrow land mass gaps seems to fit the evolutionary divergence of the genes sampled and their estimated rate as inferred by the penalized likelihood smoothing methods.

DISCUSSION

ML and Bayesian Inference Analyses of Gloxinieae Relationships—ML and Bayesian inference analyses of the phylogenetic relationships within the Gloxinieae provide a relatively well-resolved and well-supported phylogenetic hypothesis (Fig. 2). These results are generally congruent with previous analyses of these datasets using maximum parsimony (Roalson et al. 2005a). This is illustrated by the maximum parsimony bootstrap percentages mapped onto the ML phylogenetic hypothesis (Fig. 2). No branches previously considered moderately or well supported by bootstrap percentages above 70% or 95% are not present in the ML tree, and most branches with >70% bootstrap support have high or moderate Bayesian posterior probability values (Fig. 2; Roalson et al. 2005a). Additionally, several branches without parsimony bootstrap support are strongly supported by the Bayesian posterior probability values (Fig. 2). While there has been some controversy regarding the interpretation of Bayesian posterior probability values, we consider them to provide the confidence interval around each branch. Given the consistent posterior probability distributions postburnin from different start locations in multiple independent Bayesian analyses, we suggest that the posterior probability distribution of trees and parameters are adequately sampled and reflect the true parameter distribution. Therefore, as we are confident that our model choice was appropriate given the datasets, we expect that the posterior probability scores reflect the 95% confidence interval for tree branches. It has been previously noted that posterior probability values are sometimes able to provide high confidence on branches with low bootstrap percentages when internodes are short due to an apparent greater sensitivity of Bayesian PP to signal in the datasets (Alfaro et al. 2003). As with the branches also supported by high bootstrap values, we consider these branches with high posterior probability values, but low bootstrap support, to be well supported (Fig. 2).

Reconstruction of Biogeographic Patterns—Biogeographic patterns in the Gesneriaceae rarely have been explored, and, traditionally, discussions of biogeographic origins and patterns in the family have relied on association of areas of

highest diversity with an implied area of origin (Gentry 1982; Kvist and Skog 1992, 1996; Skog and Kvist 2000). No explicit biogeographic reconstructions have previously been made in the New World Gesnerioideae, but this group has been thought to have originated in northern South America due to the high diversity of lineages of the subfamily in this region and it has been suggested that lineages in Central America and the Caribbean Islands have resulted from multiple dispersal events from South America. The results presented here suggest several alternative reconstructions of the biogeographic patterns in the New World Gesnerioideae and the Gloxinieae tribe.

In the FPO and DIVA analyses of the Gesnerioideae and Gloxinieae, the reconstruction of the ancestral distribution for the Gloxinieae is entirely dependent on whether the two Central American clades form a grade leading to the rest of the Gloxinieae or are sister clades (Figs. 3, 4). Unfortunately, these nodes are not well supported in the ML and Bayesian analyses (Figs. 1, 2) and the alternative hypothesis of a monophyletic Central American clade cannot be excluded based on tests of likelihood scores using the SH test. Given the low support of one of these hypotheses over the other, it is best to consider these two topologies equally likely.

While this lack of support of one hypothesis over the other could be interpreted as uninformative, we believe an alternative perspective is more appropriate. Historically, biogeographic hypotheses have been formed entirely on the regions of highest diversity argument. These analyses suggest two equally plausible (given the data) competing biogeographic hypotheses which can be tested with additional data and sampling in the future: (1) the Gloxinieae tribe originated in Central America; or (2) the ancestor of the Gloxinieae had a widespread distribution, including both Central America and Andean/western South America. Additional gene sequences (such as low copy number nuclear genes or more variable chloroplast spacers) may help resolve weak nodes and thus narrow down which hypothesis might be most likely. It seems clear that the likelihood of the competing hypotheses is dependent on the resolution of a few nodes associated with the relationships among the two clades of predominantly Central American genera and the large and strongly supported predominantly Andean/western South American clade of Gloxinieae genera. Other lineages in this clade found in Central America clearly appear to be separate dispersal events from South America as they are nested within predominantly South American clades of species. It should be noted that while some reconstructions suggest the Gloxinieae originated in Central America, none of these analyses suggest the ancestor was restricted to Andean/western South America (Figs. 3, 4). This is notable in that while confidence in some of the nodes of interest might be low; all indications suggest that this lineage was in Central America prior to the divergence of Gloxinieae lineages that are currently extant.

Other recent phylogenetic studies of the Gloxinieae based on a combination of these and other gene regions (Smith et al. 2004a, b) have resulted in phylogenetic hypotheses that differ significantly from the phylogenetic results found here and elsewhere (Zimmer et al. 2002; Roalson et al. 2005a). This conflict has been previously found in relation to other Smith et al. studies (particularly Smith 2000b) by Zimmer et al. (2002), who noted that the exact same samples sequenced differed by as much as 4% and had a large number of am-

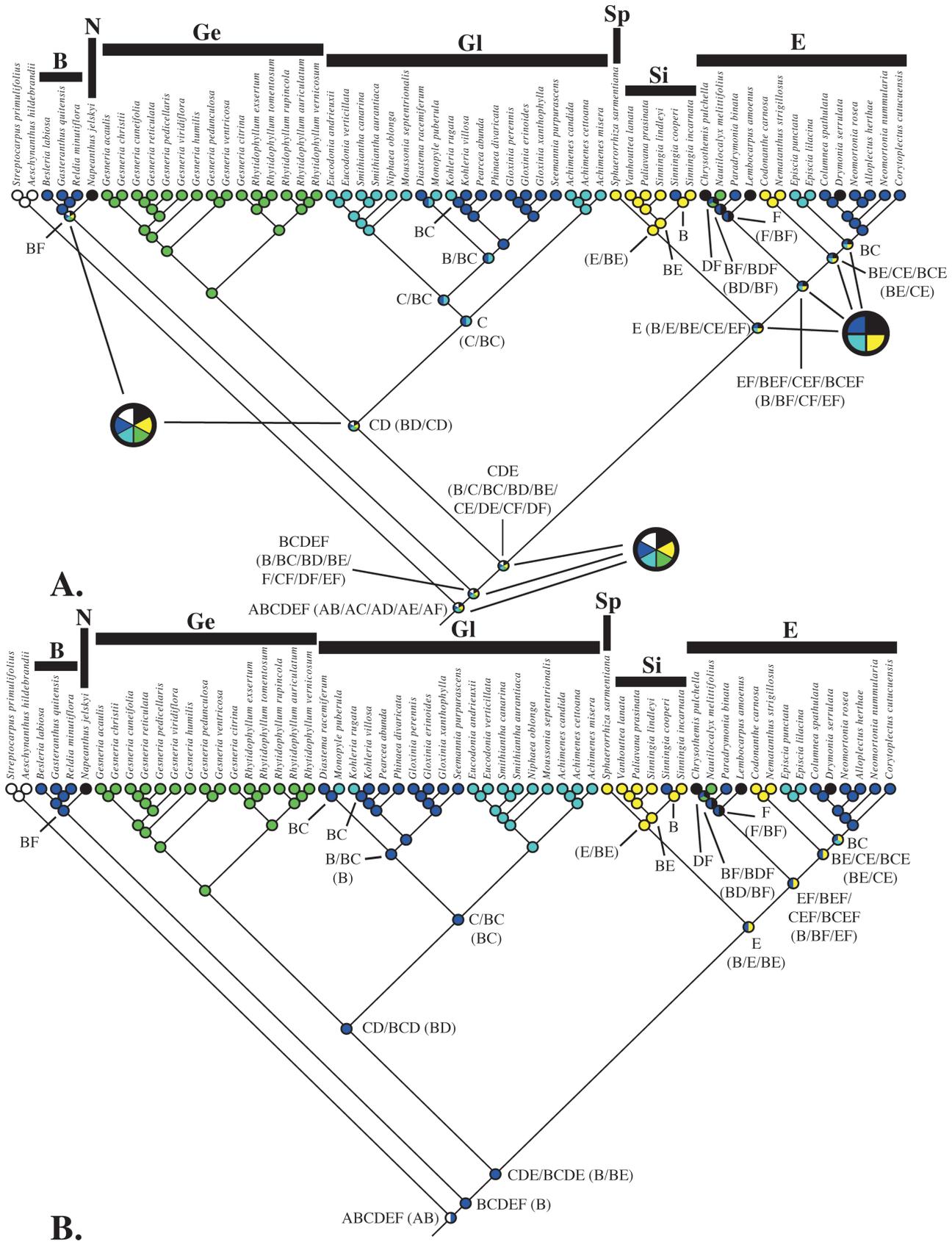


FIG. 3. FPO and DIVA optimizations of biogeographic patterns in the Gesnerioideae. Colored circles at each node represent FPO reconstructions and letters note DIVA reconstructions as follows: white & A = Old World; dark blue & B = Andean/western South America; light blue & C = Central America; green & D = Caribbean; yellow & E = southern Brazil; and black & F = Guianas. Nodes without letter designations have the same state reconstruction using DIVA and FPO. Letters in parentheses refer to DIVA reconstructions under the “maximum of two ancestral areas” constraint. Letter combinations divided by a “/” reflect alternative DIVA reconstructions. Bars above clades denote tribes abbreviated as follows: B = Beslerieae, E =

ambiguous base calls. While polymorphism has been found in ITS sequences among many plant lineages, significant polymorphism within species and individuals has not been found in Gesnerioideae (particularly Gesnerieae and Gloxinieae) ITS sequences (unpubl. data). In assessing the different placement of species in previous analyses, some taxa have been clearly misplaced in these studies, notably the placement of *Sanango* within the Gesnerieae tribe (Smith et al. 1997a; erroneous placement discussed by Weber 2004), placement of *Lembocarpus* within the Gloxinieae tribe (Smith 2001; placement clarified by Smith et al. 2004b; Roalson et al. 2005a; this paper), and placement of *Capanea grandiflora* (*Kohleria tigridia*) in the Episcieae tribe (Smith 2001; placement clarified by Roalson et al. 2005a; this paper). As with these previous issues, more recent Smith et al. (2004a, b) publications have similarly conflicting phylogenies. Particularly, the placement of *Solenophora* sister to *Niphaea*, *Koellikeria* (*Gloxinia*) *erinoides* sister to *Goyazia*, *Anodiscus* (*Gloxinia*) *xanthophyllus* sister to *Seemannia* (all in Smith et al. 2004a), and *Niphaea* sister to *Gloxinia*, *Diastema* sister to *Gesneria*, and *Phinaea* sister to *Gloxinia* (all in Smith et al. 2004b) are problematic as their placement is very different from other studies (Zimmer et al. 2002; Roalson et al. 2005a; this study), and also inconsistent among the different Smith et al. publications. As an example, *Diastema racemiferum* is placed sister to *Gesneria* in Smith et al. 2004a and sister to *Heppiella* in Smith et al. 2004b. The movement between these branches requires crossing several strongly supported branches as measured by bootstrap and Bayesian posterior probability support values, both in the Smith et al. papers as well as Roalson et al. 2005a and this study, and moving between different tribes of the Gesnerioideae. However, many of the branches in the Smith et al. (2004a, b) studies are not strongly supported, and therefore some of these differences may be related to short branches as well as possible conflicting signal in different genic regions. Smith et al. (2004b) do note significant incongruence in their datasets as measured by the partition homogeneity test. Given the lower species sampling within the Gloxinieae in these papers and the use of eight genic regions, it is unclear why there would be less clade support and different relationships from our study using two genic regions, both of which are used in the Smith et al. (2004b) analyses. Our laboratory techniques produce sequences with strong and consistent phylogenetic signal. Until the cause of the discrepancies shown by the other gene regions analyzed by Smith et al. (2004a, b) is understood, we prefer to exclude this data from our analysis.

Dating Divergences, Plate Tectonics, and Phylogenetic Patterns—Optimally, we could test the two alternative hypotheses by comparing them to estimates of the ages of ancestral nodes and the timing of continental movements. Unfortunately, there is effectively no fossil record for the entire family Gesneriaceae, let alone a much more recently diversifying Gloxinieae tribe. This allows no point for absolute date calibration in the near vicinity of the Gloxinieae tribe. Recent studies of dating divergences have used multiple calibration points outside of the lineage of interest to effectively calibrate

nodes with no known fossil record (Yoder and Yang 2004). This kind of study is possible for the Gesneriaceae, but only through analysis of a reasonable diversity within the entire Gesneriaceae as well as sampling from throughout the Lamiales. Unfortunately, this dataset does not currently exist, but it would be possible in the future to use such a dataset to test the timing implications of our competing biogeographic hypotheses. Bremer et al. (2004) have recently applied similar methods to place the stem age of the Gesneriaceae at approximately 71 MYBP, which is similar to the 65 MYBP estimate made by Raven and Axelrod (1974). Most of the estimates of the age of the Gesnerioideae did not approach this family crown maximum, given other calibration points, and the estimated subfamily crown age of 57 MYBP (Table 2; Fig. 5) seems reasonable if the major lineages of the family diverged in the late Cretaceous to early Tertiary. Additionally, the age estimates for divergence of many of the genera in the Episcieae, Gloxinieae, and Sinningieae span the Miocene and Oligocene while those for the Gesnerieae are predominantly Pliocene and late Miocene (Fig. 5). These differences and similarities in divergence of recognized genera need to be further explored with regards to vegetation patterns in Central and South America and the Caribbean during these time periods.

Alternatively, the timing of continental movements and other geological events can be used to calibrate the timing of biogeographic events (Baldwin and Sanderson 1998; Renner et al. 2000; Bossuyt and Milinkovitch 2001; Cooper et al. 2001; Lavin et al. 2001; Richardson et al. 2001; Conti et al. 2002; Morley and Dick 2003). Here we have used five different constraint sets (Table 1) and these constraints all provide similar estimates of node age across the phylogeny. Our application of the hypothesis that the movement of gesneriad lineages among South America, Central America, and the Caribbean prior to ~3 MYBP was mediated by the presence of GAARLANDIA and the proximity of the southern Central American land mass to the northwest South American microcontinent (as described by Iturralde-Vinent and MacPhee 1999) seems to fit well with our estimates of nodal ages. While the use of geological events alone is somewhat limiting for the inference of timing of dispersal, in the event of a complete absence of a fossil record, there is little else to use to infer these patterns. It should be noted that the maximal age of the clade is derived from a fossil-calibrated study (Bremer et al. 2004), and the various other calibration points do not give significantly different ages, suggesting that the application of these maximal ages or max-min ranges are not unduly influencing (or applying a circular reasoning argument to) the inferred node ages.

It should be noted that the application of the above time calibrations to particular nodes/events requires several assumptions about the mechanisms of dispersal of gesneriads. In order to use the timing of continental movements, and particularly the formation of the GAARLANDIA land bridge, we have to assume that the formation of this bridge in some way positively influenced the opportunities for dispersal of gesneriads between these two land masses.

←

Episcieae, Ge = Gesnerieae, Gl = Gloxinieae, N = Napeantheae, Si = Sinningieae, and Sp = Sphaerorrhizeae. A. Character reconstruction with a Central America grade of clades in the Gloxinieae. Twelve dispersals inferred in unconstrained analyses and 16 inferred dispersals when constrained to two ancestral areas. B. Character reconstruction with a monophyletic Central America clade in the Gloxinieae. Twelve dispersals inferred in unconstrained analyses and 15 inferred dispersals when constrained to two ancestral areas.

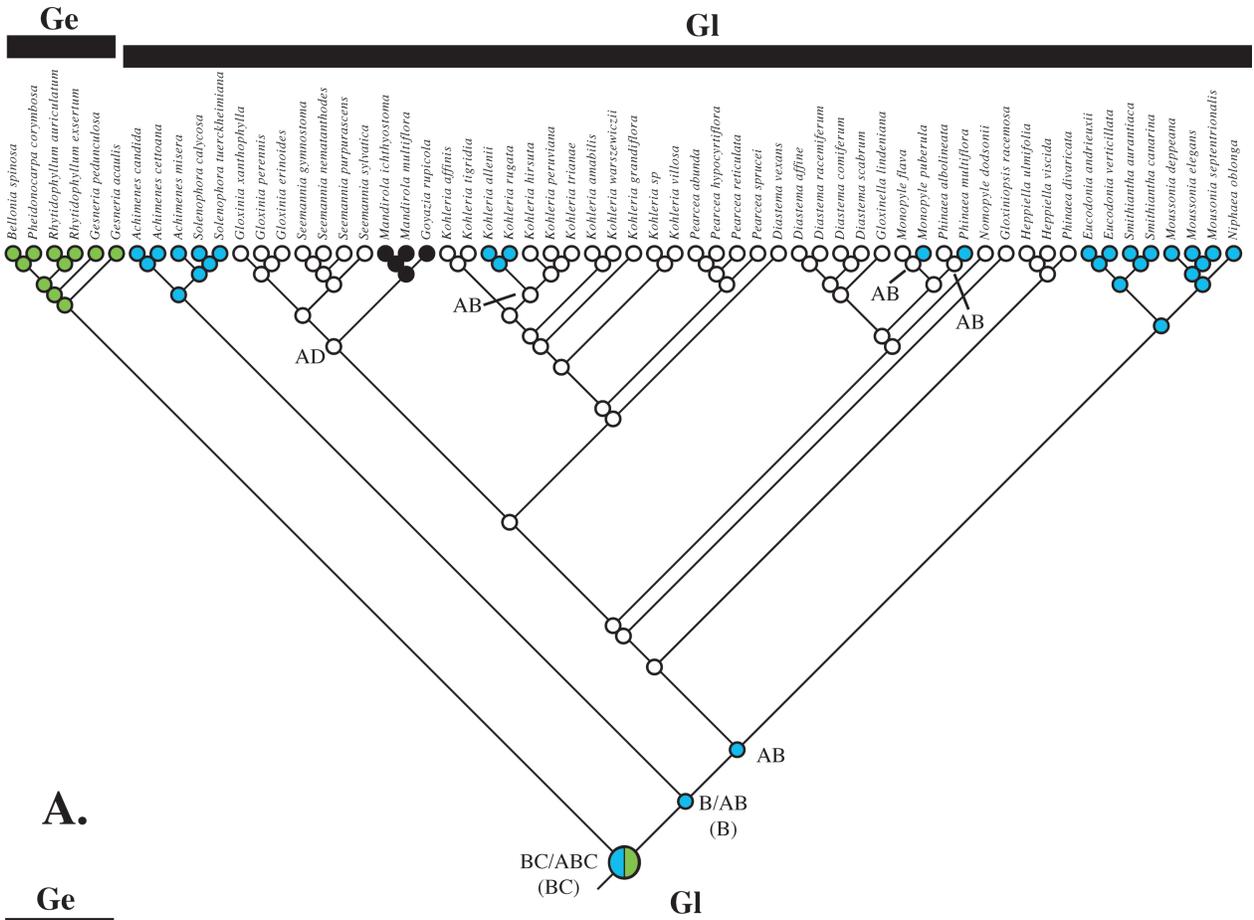


TABLE 2. Reconstruction of nodal ages using penalized likelihood. Nodes labeled 1–56 refer to nodes from Fig. 5. Details of the constraints are described in the text and Table 1. All times are given in millions of years ago (MYA). Numbers in parentheses are the minimum and maximum values for the 95% confidence interval, respectively.

Node	Constraint 56/52	Constraint 56/32	Constraint 56/8	Constraint 56/52/8	Constraint 56/32/8
1	7 (4.76, 9.04)	8.9 (5.83, 11.67)	6.9 (3.69, 9.69)	6.9 (3.71, 9.27)	6.9 (3.52, 9.8)
2	8.5 (6.36, 10.64)	11 (7.75, 13.83)	8.4 (4.91, 11.55)	8.4 (4.92, 11.08)	8.4 (4.64, 11.76)
3	11 (8.21, 13.41)	14 (10.23, 17.19)	10 (6.57, 14.33)	10 (6.49, 13.85)	10 (6.18, 14.62)
4	6.8 (4.91, 8.71)	8.6 (6.13, 11.05)	6.7 (3.87, 9.31)	6.7 (3.86, 8.98)	6.7 (3.8, 9.36)
5	9.8 (7.74, 12.06)	12 (9.77, 15.21)	9.6 (6.07, 12.99)	9.6 (6.03, 12.59)	9.6 (5.89, 13.13)
6	15 (12.85, 17.25)	19 (16.02, 21.94)	15 (10.04, 18.72)	15 (9.82, 18.38)	15 (9.65, 19.05)
7	14 (12.1, 16.42)	18 (15.09, 20.89)	14 (9.45, 17.85)	14 (9.24, 17.52)	14 (8.92, 18.28)
8	16 (14.2, 18.56)	21 (17.7, 23.7)	16 (11.09, 20.13)	16 (10.79, 19.87)	16 (11.2, 20.04)
9	1.8 (0.68, 3.04)	2.3 (0.9, 3.78)	1.8 (0.55, 3.07)	1.8 (0.53, 2.97)	1.8 (0.53, 3.13)
10	6.5 (4.74, 8.3)	8.2 (5.97, 10.49)	6.4 (3.79, 8.91)	6.4 (3.74, 8.58)	6.4 (3.54, 9.1)
11	8.9 (6.89, 10.93)	11 (8.51, 14.03)	8.8 (5.46, 11.9)	8.7 (5.41, 11.45)	8.8 (5.43, 11.91)
12	16 (13.81, 18.53)	21 (17.42, 23.54)	16 (12.76, 18.96)	16 (12.47, 18.47)	16 (12.09, 19.53)
13	17 (14.48, 18.84)	21 (17.86, 24.26)	17 (10.28, 21.6)	17 (10.07, 20.99)	17 (9.89, 21.89)
14	18 (15.49, 19.97)	23 (19.52, 25.48)	17 (13.57, 20.97)	17 (13.22, 20.5)	17 (13.57, 20.97)
15	10 (7.89, 12.57)	13 (9.67, 16.19)	10 (6.35, 13.55)	9.9 (6.24, 13.08)	10 (5.97, 13.85)
16	13 (11.05, 15.73)	17 (13.72, 20.12)	13 (8.77, 17.25)	13 (8.66, 16.62)	13 (8.23, 17.67)
17	18 (16.06, 20.78)	23 (19.89, 26.65)	18 (12.67, 23.03)	18 (12.41, 22.37)	18 (12.74, 22.98)
18	0.72 (0, 1.73)	0.94 (0, 2.19)	0.72 (0, 1.75)	0.72 (0, 1.67)	0.72 (0, 1.75)
19	2.5 (0.93, 3.97)	3.3 (1.23, 5.03)	2.5 (0.61, 4.17)	2.5 (0.6, 4)	2.5 (0.65, 4.17)
20	4.1 (2.82, 5.34)	5.2 (3.64, 6.8)	4.1 (2.44, 5.6)	4 (2.4, 5.36)	4.1 (2.5, 5.58)
21	3.9 (2.17, 5.33)	5 (2.73, 6.85)	3.9 (1.59, 5.59)	3.9 (1.59, 5.39)	3.9 (1.45, 5.69)
22	5.4 (3.86, 6.66)	7 (4.94, 8.62)	5.4 (3.3, 7.18)	5.4 (3.22, 6.82)	5.4 (3.3, 7.18)
23	6.1 (4.3, 7.42)	7.8 (5.54, 9.38)	6.1 (3.81, 7.77)	6 (3.82, 7.38)	6.1 (3.94, 7.7)
24	3.4 (1.94, 4.86)	4.4 (2.41, 6.29)	3.4 (1.66, 5.02)	3.4 (1.62, 4.82)	3.4 (1.54, 5.1)
25	7.2 (4.95, 8.75)	9.3 (6.34, 11.14)	7.2 (4.66, 9.06)	7.1 (4.62, 8.62)	7.2 (4.66, 9.06)
26	0.35 (0, 0.79)	0.45 (0, 1.02)	0.35 (0, 0.96)	0.35 (0, 0.93)	0.35 (0, 0.96)
27	5.7 (4.14, 7.54)	7.4 (5.33, 9.57)	5.7 (3.4, 8.16)	5.7 (3.38, 7.74)	5.7 (3.2, 8.32)
28	2.9 (1.56, 4.28)	3.7 (2.1, 5.34)	2.9 (1.25, 4.53)	2.8 (1.26, 4.3)	2.9 (1.16, 4.6)
29	4.2 (2.67, 5.75)	5.4 (3.32, 7.48)	4.2 (2.15, 6.19)	4.1 (2.24, 5.8)	4.2 (1.99, 6.31)
30	6.3 (4.64, 8.16)	8.1 (5.9, 10.46)	6.3 (3.9, 8.78)	6.3 (3.86, 8.34)	6.3 (3.69, 8.93)
31	8.5 (6.24, 10.68)	11 (7.84, 13.76)	8.5 (5.22, 11.54)	8.4 (5.19, 10.95)	8.5 (5.43, 11.43)
32	26 (22.47, 29.47)	33 (28.45, 36.97)	26 (17.74, 33.38)	26 (17.45, 31.97)	26 (17.76, 33.36)
33	9.3 (7.28, 12.12)	12 (8.96, 15.4)	9.4 (5.71, 13.51)	9.3 (5.71, 12.79)	9.4 (5.46, 13.7)
34	9.9 (7.75, 11.95)	13 (9.55, 15.35)	9.9 (5.61, 13.69)	9.9 (5.57, 13.09)	9.9 (5.61, 13.69)
35	11 (8.96, 12.92)	13 (10.99, 16.51)	11 (6.94, 14.74)	11 (6.92, 13.96)	11 (6.96, 14.72)
36	13 (10.89, 15.73)	16 (13.35, 20.07)	13 (8.4, 17.96)	13 (8.38, 17.02)	13 (8.4, 17.96)
37	15 (12.24, 18.16)	19 (15.16, 23.08)	15 (9.48, 20.72)	15 (9.49, 19.57)	15 (9.46, 20.74)
38	18 (14.34, 22.18)	23 (17.85, 28.17)	18 (10.99, 25.27)	18 (11.05, 23.81)	18 (10.44, 25.68)
39	21 (18.18, 24.3)	27 (22.45, 31.01)	21 (13.69, 28.37)	21 (13.65, 26.81)	21 (13.33, 28.65)
40	8.3 (5.68, 11.76)	11 (7.14, 14.86)	8.3 (4.65, 12.69)	8.3 (4.63, 12.07)	8.3 (4.45, 12.85)
41	23 (20.08, 26.64)	30 (24.5, 34.42)	23 (15.2, 31.24)	23 (15.18, 29.42)	23 (14.75, 31.59)
42	12 (9.44, 14.92)	15 (11.59, 18.91)	12 (7.35, 16.87)	12 (7.38, 15.94)	12 (6.97, 17.17)
43	19 (16.12, 22.44)	24 (19.82, 28.5)	19 (12.3, 26.02)	19 (12.37, 24.49)	19 (11.63, 26.55)
44	25 (22.78, 28.46)	32 (27.58, 36.82)	26 (16.71, 34.39)	25 (16.93, 32.13)	26 (17.08, 34.12)
45	28 (25.39, 30.31)	35 (30.54, 39.58)	28 (18.55, 36.99)	28 (18.75, 34.59)	28 (18.9, 36.74)
46	14 (11.06, 15.82)	17 (13.63, 20.39)	14 (8.31, 18.47)	14 (8.42, 17.3)	14 (8.31, 18.47)
47	15 (12.02, 17.06)	18 (14.82, 21.82)	15 (9.26, 19.58)	14 (9.34, 18.38)	15 (8.63, 20.07)
48	17 (14.38, 19.02)	21 (17.56, 24.48)	17 (10.79, 22.35)	17 (10.8, 21.04)	17 (10.05, 22.93)
49	18 (15.67, 20.55)	23 (19.14, 26.46)	18 (11.99, 24.03)	18 (11.98, 22.66)	18 (12.49, 23.81)
50	32 (28.7, 33.58)	40 (33.86, 44.42)	32 (18.21, 42.93)	32 (18.33, 40.25)	32 (19.18, 42.34)
51	32 (30.07, 34.03)	41 (35.66, 45.26)	32 (21.94, 42.1)	32 (22.01, 39.45)	32 (22.45, 42.33)
52	35 (35, 35)	45 (39.64, 48.96)	35 (24.35, 45.79)	35 (24.46, 42.74)	35 (25.09, 45.73)
53	15 (12.07, 18.47)	19 (15.08, 23.08)	15 (10.09, 20.41)	15 (10.05, 19.29)	15 (9.5, 20.86)
54	17 (13.24, 20.88)	21 (16.62, 25.98)	17 (10.86, 23.1)	17 (10.82, 21.86)	17 (10.18, 23.62)
55	52 (43.68, 60.44)	65 (57.22, 71.62)	52 (33.63, 69.51)	52 (33.65, 65.45)	52 (31.09, 71.49)
56	56.89 (51.04, 63.88)	71 (70.02, 71.78)	57.23 (39.1, 74.86)	56.64 (38.88, 70.76)	57.23 (40.23, 74.15)

←

FIG. 4. FPO and DIVA optimizations of biogeographic patterns in the Gloxinieae. Colored circles at each node represent FPO reconstructions and letters note DIVA reconstructions as follows: white & A = Andean/western South America; blue & B = Central America; green & C = Caribbean; and black & D = southern Brazil. Nodes without letter designations have the same state reconstruction using DIVA and FPO. Letters in parentheses refer to DIVA reconstructions under the “maximum of two ancestral areas” constraint. Letter combinations divided by a “/” reflect alternative DIVA reconstructions. Bars above clades denote tribes abbreviated as follows: Ge = Gesnerieae and Gl = Gloxinieae. A. Character reconstruction with a Central America grade of clades in the Gloxinieae. Five dispersals inferred in unconstrained analyses and five inferred dispersals when constrained to two ancestral areas. B. Character reconstruction with a monophyletic Central America clade in the Gloxinieae. Four dispersals were inferred in the unconstrained analyses, and five when constrained to two ancestral areas.

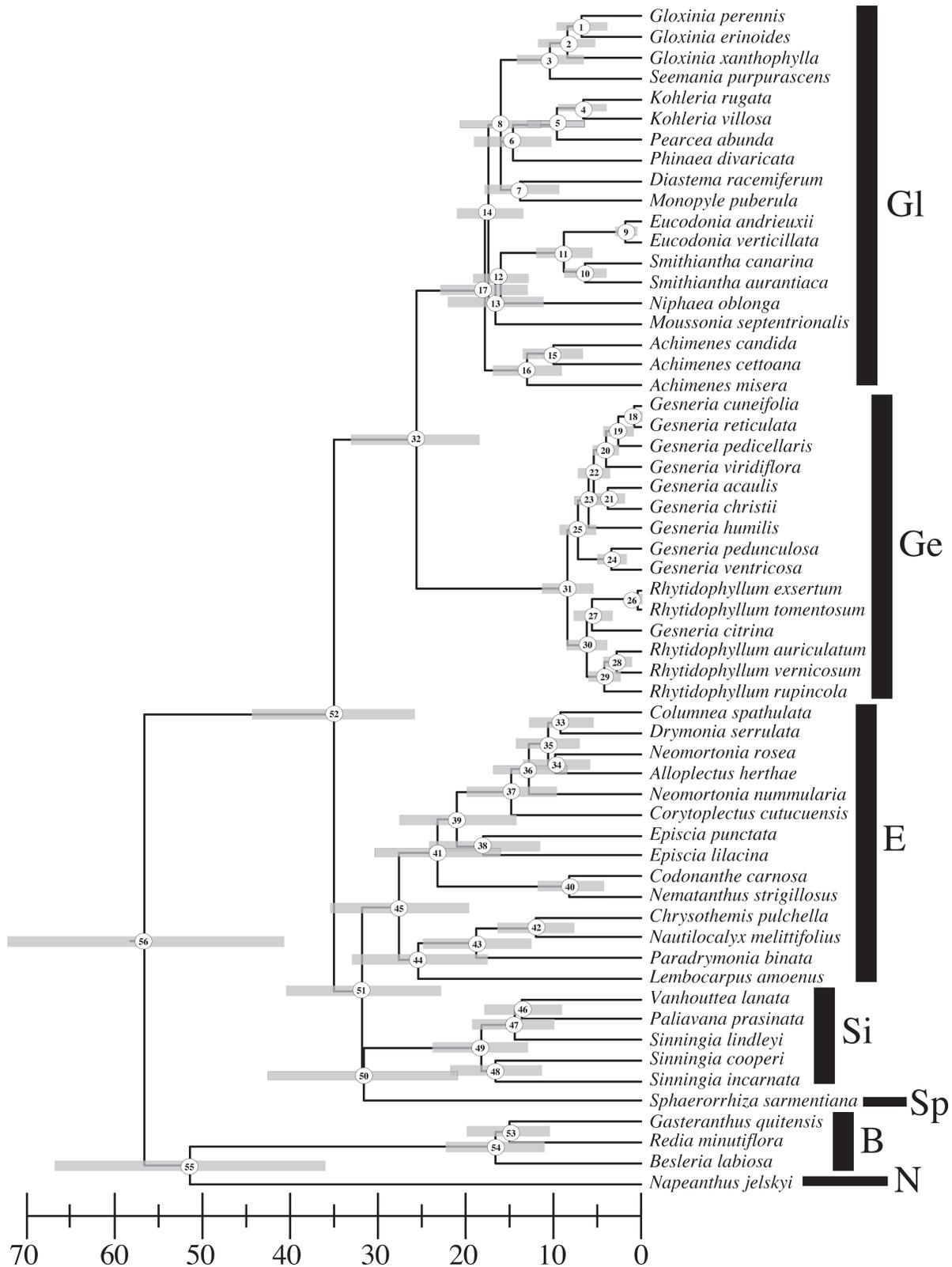


FIG. 5. Chronogram reflecting divergence times in millions of years ago (MYA) using the 56/52/8 constraints. Circled numbers refer to node divergence calculations listed in Table 2. Shaded bars reflect the bootstrap 95% confidence interval described in Table 2. Bars to the right of clades denote tribes abbreviated as follows: B = Beslerieae, E = Episcieae, Ge = Gesnerieae, Gl = Gloxinieae, N = Napeantheae, Si = Sinningieae, and Sp = Sphaerorrhizeae.

Nothing definitive is known about mechanisms of dispersal in New World gesneriads and circumstantial inferences of possible dispersal vectors suggest that there are several potential vectors including wind, animals (both through

consumption and passive dispersal), and water. It is not clear how each of these dispersal vectors would be influenced by the formation of a land bridge. Endozoochory seems unlikely for many Gesneriaceae species given the small seed size in

the family and the lack of a hard seed coat. If shorter distance mechanisms such as splash cups and ant dispersal were dominant dispersal types of ancestral lineages, we might expect that dispersal between the Caribbean Islands and South America would be positively influenced by GAARLANDIA. Alternatively, if wind dispersal or long distance dispersal passively or internally by birds were the more dominant dispersal mechanisms, we might expect a lack of correlation of long distance dispersal among Caribbean islands, Central America, and South America and the formation of GAARLANDIA or other island stepping stone formations.

So what do we know about the distribution of dispersal mechanisms in the Gesneriaceae? Several authors have provided anecdotal hypotheses of dispersal mechanisms in different lineages in the family. In the Old World, Burt (1970, 1976), Carlquist (1970), Sakai et al. (1995), Denduangboripant and Cronk (2000), Denduangboripant et al. (2001), Mendum et al. (2001), Kiehn (2001), Price and Wagner (2004), and Cronk et al. (2005) have discussed dispersal mechanisms primarily in regard to interisland dispersal of *Aeschynanthus* and *Cyrtandra* among southeast Asian and Pacific island landmasses. They particularly note that the coma of filiform appendages in some *Aeschynanthus* and the white fleshy fruits of the Pacific island lineages of *Cyrtandra* might play roles in their success in dispersal through wind and putative bird endozoochory, respectively. Burt (1970, 1976) also noted the likely dispersal of understory herbs through water dispersal mechanisms, such as splash cups and adherence of the tiny seeds to animals.

In the New World, evidence for dispersal is largely associated with the genera *Columnnea*, *Gasteranthus*, *Gesneria*, and *Kohleria* (Stearn 1969; Skog and Kvist 2000; Skog 1976; and Kvist and Skog 1992; respectively). Fruits in Jamaican *Columnnea* species are presumably dispersed by bird consumption (Stearn 1969), although others have suggested these seeds might as likely be dispersed by ants (attributed to Morley by Stearn 1969). *Gasteranthus* generally have fleshy capsules that split to reveal a mass of seeds. The dispersal mechanism for these seeds is unclear, but rain wash in the very wet habitats where *Gasteranthus* is found and accidental sticking of the tiny seeds to passing animals are the most likely contributors to seed dispersal (Skog and Kvist 2000). Given the much more widespread distribution of *Gasteranthus pansamalanus* compared to most other *Gasteranthus* species, Skog and Kvist (2000) suggested these seeds might be dispersed by bird consumption of the berry-like capsules.

Skog (1976) outlined three likely mechanisms of dispersal for *Gesneria* in his revision of the Gesnerieae. He considered that transport of the small seeds in dry capsules would only be carried incidentally by large animals, but that invertebrates and particularly ants might be important dispersers. Plants growing near or in streams might likely have seeds carried downstream by water in addition to the splash cup dispersal found in some species. Finally, dispersal by wind seems likely to be a major mechanism of dispersal in *Gesneria*. He further notes that dispersal by animals and water seem to be primarily short distance dispersal mechanisms with dispersal by wind the only opportunity for long distance dispersal of *Gesneria* seeds among the different Caribbean islands. *Kohleria* appears to include species with both wind and animal dispersal of seeds (Kvist and Skog 1992). The putatively wind-dispersed species are generally quite widespread

and have small dust-like seeds in dehiscent dry capsules. Other species have seeds presented in glutinous seed masses that are presumably dispersed by animals, including insects that may prey on seed masses and become covered in the process.

An additional process possibly involved in dispersal of gesneriads in the New World is hurricanes. Given the prevailing patterns of hurricane movement – from the southeast to the northwest – this seems to be a potential contributor to plant movement. It could be that we do not see adaptation for long distance seed dispersal in the gesneriads for a reason, that is, selection has been for shorter distance movement, which is the more common dispersal pattern for population distributions, and this is why most species of New World Gesneriaceae have narrow range distributions. On the rare occasions when hurricanes are strong enough and follow the necessary trajectory, however, species might be moved with little adaptation involved, as hurricane-force winds might carry not only seeds, but fruits or large plant fragments as well. So if hurricane-mediated dispersal has been involved in moving gesneriads in the past, why do we not see more of the northeastern South American gesneriad genera in the Caribbean? One potential explanation for this is that while hurricane-mediated dispersal is possible, it is an extremely rare event and/or the current large Caribbean land masses are too far for this to be an effective mechanism. In this case, the presence of the GAARLANDIA bridge might have allowed for more effective hurricane-mediated dispersal.

If GAARLANDIA and the Central American land masses did positively influence major dispersal events, it might be expected that some of the midrange dispersal mechanisms including wind (hurricane or otherwise) and ectozoochory might have been important in these dispersal events. Given the rarity of widely distributed species in the Gesnerioideae, it seems more likely that shorter dispersal distances and the presence of land bridges played a role in these movements. The examples given here of potential dispersal mechanisms and fruit/seed types covers most of the major variants we see in the Gesnerioideae. Which types of fruit/seed types were most likely associated with the branches where we think major dispersal events were occurring needs further exploration, but given that the Gesnerieae and Gloxinieae tribes are dominated by dry capsules, it seems likely that our inferences of dispersal mechanisms are accurate.

In conclusion, the phylogenetic and biogeographic hypotheses presented here further support a reasonably resolved phylogeny of Gesneriaceae tribe Gloxinieae and suggest that the ancestor of the Gloxinieae tribe was either restricted to Central America or broadly distributed through Central America and western/Andean South America. Further, it appears likely that the Greater Antilles/Aves Ridge landbridge played a role in the movement of the Gesnerieae and Gloxinieae tribes from South America to Central America and the Caribbean, with later back dispersals to South America in the Gloxinieae. While sampling within tribe Episceae was relatively low in these analyses, Fig. 5 suggests this tribe may have diversified earlier (or had less extinction) than the Gloxinieae or Gesnerieae lineages, and this should be further explored. The relatively young age of the extant Gesnerieae should also be further explored, as this may be associated with greater extinction rates in a lineage restricted to islands.

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APPENDIX 1. Samples used in phylogenetic analyses and their associated GenBank numbers (ITS/*trnL-F*/*trnE-T*) with genera and species listed alphabetically. All voucher information for these specimens is previously published in Zimmer et al. (2002) and Roalson et al. (2005a), but here follow the classification of Roalson et al. (2005b).

Achimenes candida Lindl., AY047065 / AY047124 / AY047183; *A. cetoana* H.E.Moore, AY047066 / AY047125 / AY047184; *A. misera* Lindl., AY047067 / AY047126 / AY047185; *Aeschynanthus hildebrandii* Hemsl., AY047040 / AY047099 / AY047158; *Alloplectus herthae* Mansf. (previously identified as *Alloplectus bolivianus* (Britton) Wiehler), AY047097 / AY047156 / AY047215; *Bellonia spinosa* Swartz, AY702350 / AY702394 / —; *Besleria labiosa* Hanst., AY047041 / AY047100 / AY047159; *Chrysothemis pulchella* (Donn ex Sims) Decne., AY047085 / AY047144 / AY047203; *Codonanthe carnosa* (Gardner) Hanst., AY047088 / AY047147 / AY047206; *Columnea spathulata* Mansf., AY047092 / AY047151 / AY047210; *Corytoplectus cutucuensis* Wiehler, AY047094 / AY047153 / AY047212; *Diastema affine* Fritsch, AY702353 / AY702397 / —; *D. comiferum* (DC.) Benth. ex Walp., AY702354 / AY702398 / —; *D. racemiferum* Benth., AY047069 / AY047128 / AY047187; *D. scabrum* (Poepp.) Benth. ex Walp., AY702356 / AY702400 / —; *D. vexans* H.E.Moore, AY702357 / AY702401 / —; *Drymonia serrulata* (Jacq.) Mart., AY047093 / AY047152 / AY047211; *Episcia lilacina* Hanst., AY047091 / AY047150 / AY047209; *E. punctata* (Lindl.) Hanst., AY047090 / AY047149 / AY047208; *Eucondonia andrieuxii* (DC.) Wiehler, AY047060 / AY047119 / AY047178; *E. verticillata* (M.Martens & Galeotti) Wiehler, AY047061 / AY047120 / AY047179; *Gasteranthus quitensis* Benth., AY047042 / AY047101 / AY047160; *Gesneria acaulis* L., AY047045 / AY047104 / AY047163; *G. christii* Urb., AY047046 / AY047105 / AY047164; *G. citrina* Urb., AY047054 / AY047113 / AY047172; *G. cuneifolia* (DC.) Fritsch, AY047047 / AY047106 / AY047165; *G. humilis* L., AY047051 / AY047110 / AY047169; *G. pedicellaris* Alain, AY047049 / AY047108 / AY047167; *G. pedunculosa* (DC.) Fritsch, AY047052 / AY047111 / AY047170; *G. reticulata* (Griseb.) Urb., AY047048 / AY047107 / AY047166; *G. ventricosa* Sw., AY047053 / AY047112 / AY047171; *G. viridiflora* (Decne.) Kuntze ssp. *sintenisii* (Urb.) L.E.Skog, AY047050 / AY047109 / AY047168; *Gloxinia erimoides* (DC.) E.H.Roalson & J.K.Boggan, AY047073 / AY047132 / AY047191; *G. perennis* (L.) Fritsch, AY047071 / AY047130 / AY047189; *G. xanthophylla* (Poepp.) E.H.Roalson & J.K.Boggan, AY047074 / AY047133 / AY047192; *Gloxinella lindeniana* (Regel) E.H.Roalson & J.K.Boggan, AY702361 / AY702405 / —; *Gloxiniopsis racemosa* (Benth.) E.H.Roalson & J.K.Boggan, AY702364 / AY702407 / —; *Goyazia rupicola* Taubert, AY702366 / AY702409 / —; *Heppiella ulmifolia* (Kunth) Hanst., AY702369 / AY702412 / —; *H. viscida* (Lindl. & Paxt.) Fritsch, AY702370 / AY702413 / —; *Kohleria affinis* (Fritsch) E.H.Roalson & J.K.Boggan, AY702351 / AY702395 / —; *K. allenii* Standl. & L.O.Wms., AY702371 / AY702414 / —; *K. amabilis* (Planch. & Linden) Fritsch, AY702372 / AY702415 / —; *K. grandiflora* L.P.Kvist & L.E.Skog, AY702373 / AY702416 / —; *K. hirsuta* (Kunth) Regel, AY702374 / AY702417 / —; *K. peruviana* Fritsch, AY702375 / AY702418 / —; *K. rugata* (Scheidw.) L.P.Kvist & L.E.Skog, AY047075 / AY047134 / AY047193; *K. sp. nov.* [2446], AY702376 / AY702419 / —; *K. tigridia* (J.H.Ohrend.) E.H.Roalson & J.K.Boggan, AY702352 / AY702396 / —; *K. trianae* (Regel) Hanst., AY702377 / AY702420 / —; *K. villosa* (Fritsch) Wiehler, AY047076 /

AY047135 / AY047194; *K. warszewiczii* (Regel) Hanst., AY702379 / AY702422 / —; *Lembocarpus amoenus* Leeuwenb., AY702380 / AY702423 / —; *Mandirola* sp. aff. *ichthyostoma* (Gardner) B.C.Seem. ex Hanst., AY702360 / AY702404 / —; *M. multiflora* (Gardner) Decne., AY702363 / — / —; *Monopyle flava* L.E.Skog, AY702381 / AY702424 / —; *M. puberula* C.V.Morton, AY047070 / AY047129 / AY047188; *Moussonia depeana* (Schlechtend. & Cham.) Hanst., AY702383 / AY702426 / —; *M. elegans* Decne., AY702384 / AY702427 / —; *M. septentrionalis* (Denham) Wiehler, AY047068 / AY047127 / AY047186; *Napeanthus jelskyi* Fritsch, AY047044 / AY047103 / AY047162; *Nautilocalyx melittifolius* (L.) Wiehler, AY047086 / AY047145 / AY047204; *Nematanthus strigillosus* (Mart.) H.E.Moore, AY047089 / AY047148 / AY047207; *Neomortonia nummularia* (Hanst.) Wiehler, AY047096 / AY047155 / AY047214; *N. rosea* Wiehler, AY047095 / AY047154 / AY047213; *Niphaea oblonga* Lindl., AY047064 / AY047123 / AY047182; *Nomopyle dodsonii* (Wiehler) E.H.Roalson & J.K.Boggan, AY702358 / AY702402 / —; *Paliavana prasinata* (Ker Gawl.) Fritsch, AY047081 / AY047140 / AY047199; *Paradrymonia binata* Wiehler, AY047087 / AY047146 / AY047205; *Pearcea abunda* (Wiehler) L.P.Kvist & L.E.Skog, AY047077 / AY047136 / AY047195; *P. hypocyrtiflora* (Hook.f.) Regel, AY702385 / AY702428 / —; *P. reticulata* (Fritsch) L.P.Kvist & L.E.Skog, AY702386 / AY702429 / —; *P. sprucei* (Britton) L.P.Kvist & L.E.Skog, AY702387 / AY702430 / —; *Pheidonocarpa corymbosa* (Swartz) L.E.Skog, AY702388 / AY702431 / —; *Phinaea albolineata* (Hook.) Benth. ex Hemsl., AY702389 / AY702432 / —; *P. divaricata* (Poepp.) Wiehler (previously published as *P. ecuadorana* Wiehler, which is now considered a synonym of *P. divaricata*), AY047078 / AY047137 / AY047196; *P. multiflora* C.V.Morton, AY702390 / AY702433 / —; *Reldia minutiflora* (L.E.Skog) L.P.Kvist & L.E.Skog var. *minutiflora*, AY047043 / AY047102 / AY047161; *Rhytidophyllum auriculatum* Hook., AY047058 / AY047117 / AY047176; *R. exsertum* Griseb., AY047055 / AY047114 / AY047173; *R. rupicola* (C.Wright) C.V.Morton, AY047057 / AY047116 / AY047175; *R. tomentosum* (L.) Mart., AY047056 / AY047115 / AY047174; *R. vernicosum* Urb. & Ekman, AY047059 / AY047118 / AY047177; *Seemannia gymnostoma* (Griseb.) M.Tours., AY702359 / AY702403 / —; *S. nematanthodes* (Kuntze) J.Schum., AY702362 / AY702406 / —; *S. purpurascens* Rusby [B], AY047072 / AY047131 / AY047190; *S. sylvatica* (Kunth) Hanst., AY702365 / AY702408 / —; *Sinningia cooperi* (Paxton) Wiehler, AY047082 / AY047141 / AY047200; *S. incarnata* (Aubl.) Denham, AY047083 / AY047142 / AY047201; *S. lindleyi* Schauer, AY047084 / AY047143 / AY047202; *Smithiantha aurantiaca* Wiehler, AY047063 / AY047122 / AY047181; *S. canarina* Wiehler, AY047062 / AY047121 / AY047180; *Solenophora calycosa* J.D.Sm., AY702392 / AY702435 / —; *S. tuerkheimiana* J.D.Sm., AY702393 / AY702436 / —; *Sphaerorrhiza sarmentiana* (Gardner ex Hook.) E.H.Roalson & J.K.Boggan, AY047079 / AY047138 / AY047197; *Streptocarpus primulifolius* Gand., AY047039 / AY047098 / AY047157; *Vanhouttea lanata* Fritsch, AY047080 / AY047139 / AY047198.

APPENDIX 2. Gesnerioideae biogeographic distributions used in coding Fitch Parsimony Optimization (FPO) and DIVA data matrices. Geographic abbreviations are as follows: C. Am. = Central America; C. I. = Caribbean Islands; A./W. S. Am. = Andean/Western South America; S. B. = Southern Brazil; G. = Guianas; and O. W. = Old World.

Species	Geographic Distribution
<i>Achimenes candida</i>	C. Am.
<i>Achimenes cettoana</i>	C. Am.
<i>Achimenes misera</i>	C. Am.
<i>Aeschynanthus hildebrandii</i>	O. W.
<i>Alloplectus herthae</i>	A./W. S. Am.
<i>Besleria labiosa</i>	A./W. S. Am.
<i>Chrysothemis pulchella</i>	G.
<i>Codonanthe carnosa</i>	S. B.
<i>Columnea spathulata</i>	A./W. S. Am.
<i>Corytoplectus cutucuensis</i>	A./W. S. Am.
<i>Diastema racemiferum</i>	A./W. S. Am.
<i>Drymonia serrulata</i>	G.
<i>Episcia lilacina</i>	C. Am.
<i>Episcia punctata</i>	C. Am.
<i>Eucodonia andrieuxii</i>	C. Am.
<i>Eucodonia verticillata</i>	C. Am.
<i>Gasteranthus quitensis</i>	A./W. S. Am.
<i>Gesneria acaulis</i>	C. I.
<i>Gesneria christii</i>	C. I.

APPENDIX 2. Continued.

Species	Geographic Distribution
<i>Gesneria citrina</i>	C. I.
<i>Gesneria cuneifolia</i>	C. I.
<i>Gesneria humilis</i>	C. I.
<i>Gesneria pedicellaris</i>	C. I.
<i>Gesneria pedunculosa</i>	C. I.
<i>Gesneria reticulata</i>	C. I.
<i>Gesneria ventricosa</i>	C. I.
<i>Gesneria viridiflora</i>	C. I.
<i>Gloxinia erinoides</i>	A./W. S. Am.
<i>Gloxinia perennis</i>	A./W. S. Am.
<i>Gloxinia xanthophylla</i>	A./W. S. Am.
<i>Kohleria rugata</i>	C. Am.
<i>Kohleria villosa</i>	A./W. S. Am.
<i>Lembocarpus amoenus</i>	G.
<i>Monopyle puberula</i>	C. Am.
<i>Moussonia septentrionalis</i>	C. Am.
<i>Napeanthus jelskyi</i>	G.
<i>Nautilocalyx melittifolius</i>	C. I.
<i>Nematanthus strigillosus</i>	S. B.
<i>Neomortonia nummularia</i>	A./W. S. Am.
<i>Neomortonia rosea</i>	A./W. S. Am.
<i>Niphaea oblonga</i>	C. Am.
<i>Paliavana prasinata</i>	S. B.
<i>Paradrymonia binata</i>	A./W. S. Am.
<i>Pearcea abunda</i>	A./W. S. Am.
<i>Phinaea divaricata</i>	A./W. S. Am.
<i>Reldia minutiflora</i>	A./W. S. Am.
<i>Rhytidophyllum auriculatum</i>	C. I.
<i>Rhytidophyllum exsertum</i>	C. I.
<i>Rhytidophyllum rupicola</i>	C. I.
<i>Rhytidophyllum tomentosum</i>	C. I.
<i>Rhytidophyllum vernicosum</i>	C. I.
<i>Seemannia purpurascens</i>	A./W. S. Am.
<i>Sinningia cooperi</i>	A./W. S. Am.
<i>Sinningia incarnata</i>	S. B.
<i>Sinningia lindleyi</i>	S. B.
<i>Smithiantha aurantiaca</i>	C. Am.
<i>Smithiantha canarina</i>	C. Am.
<i>Sphaerorrhiza sarmentiana</i>	S. B.
<i>Streptocarpus primulifolius</i>	O. W.
<i>Vanhouttea lanata</i>	S. B.

APPENDIX 3. Gloxinieae biogeographic distributions used in coding Fitch Parsimony Optimization (FPO) and DIVA data matrices. Geographic abbreviations are as follows: C. Am. = Central America; C.I. = Caribbean Islands; A./W. S. Am. = Andean/Western South America; and S. B. = Southern Brazil.

Species	Geographic Distribution
<i>Achimenes candida</i>	C. Am.
<i>Achimenes cettoana</i>	C. Am.
<i>Achimenes misera</i>	C. Am.
<i>Bellonia spinosa</i>	C.I.
<i>Diastema affine</i>	A./W. S. Am.
<i>Diastema comiferum</i>	A./W. S. Am.
<i>Diastema racemiferum</i>	A./W. S. Am.
<i>Diastema scabrum</i>	A./W. S. Am.
<i>Diastema vexans</i>	A./W. S. Am.
<i>Eucodonia andrieuxii</i>	C. Am.
<i>Eucodonia verticillata</i>	C. Am.
<i>Gesneria acaulis</i>	C.I.
<i>Gesneria pedunculosa</i>	C.I.
<i>Gloxinia erinoides</i>	A./W. S. Am.
<i>Gloxinia perennis</i>	A./W. S. Am.
<i>Gloxinia xanthophylla</i>	A./W. S. Am.
<i>Gloxinella lindemiana</i>	A./W. S. Am.
<i>Gloxiniopsis racemosa</i>	A./W. S. Am.

APPENDIX 3. Continued.

Species	Geographic Distribution
<i>Goyazia rupicola</i>	S. B.
<i>Heppiella ulmifolia</i>	A./W. S. Am.
<i>Heppiella viscida</i>	A./W. S. Am.
<i>Kohleria affinis</i>	A./W. S. Am.
<i>Kohleria tigridia</i>	A./W. S. Am.
<i>Kohleria allenii</i>	C. Am.
<i>Kohleria amabilis</i>	A./W. S. Am.
<i>Kohleria grandiflora</i>	A./W. S. Am.
<i>Kohleria hirsuta</i>	A./W. S. Am.
<i>Kohleria peruviana</i>	A./W. S. Am.
<i>Kohleria rugata</i>	C. Am.
<i>Kohleria</i> sp. nov.	A./W. S. Am.
<i>Kohleria trianae</i>	A./W. S. Am.
<i>Kohleria villosa</i>	A./W. S. Am.
<i>Kohleria warszewiczii</i>	A./W. S. Am.
<i>Mandirola ichthyostoma</i>	S. B.
<i>Mandirola multiflora</i>	S. B.
<i>Monopyle flava</i>	A./W. S. Am.
<i>Monopyle puberula</i>	C. Am.
<i>Moussonia deppeana</i>	C. Am.
<i>Moussonia elegans</i>	C. Am.

APPENDIX 3. Continued.

Species	Geographic Distribution
<i>Moussonia septentrionalis</i>	C. Am.
<i>Niphaea oblonga</i>	C. Am.
<i>Nomopyle dodsonii</i>	A./W. S. Am.
<i>Pearcea abunda</i>	A./W. S. Am.
<i>Pearcea hypocyrtiflora</i>	A./W. S. Am.
<i>Pearcea reticulata</i>	A./W. S. Am.
<i>Pearcea sprucei</i>	A./W. S. Am.
<i>Pheidonocarpa corymbosa</i>	C.I.
<i>Phinaea albolineata</i>	A./W. S. Am.
<i>Phinaea divaricata</i>	A./W. S. Am.
<i>Phinaea multiflora</i>	C. Am.
<i>Rhytidophyllum auriculatum</i>	C.I.
<i>Rhytidophyllum exsertum</i>	C.I.
<i>Seemannia gymnostoma</i>	A./W. S. Am.
<i>Seemannia nematanthoides</i>	A./W. S. Am.
<i>Seemannia purpurascens</i>	A./W. S. Am.
<i>Seemannia sylvatica</i>	A./W. S. Am.
<i>Smithiantha aurantiaca</i>	C. Am.
<i>Smithiantha canarina</i>	C. Am.
<i>Solenophora calycosa</i>	C. Am.
<i>Solenophora tuerckheimiana</i>	C. Am.