American Journal of Botany 89(2): 296–311. 2002.

PHYLOGENETIC RELATIONSHIPS IN THE GESNERIOIDEAE (GESNERIACEAE) BASED ON NRDNA ITS AND CPDNA *TRNL-F* AND *TRNE-T* SPACER REGION SEQUENCES[1]

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The Gesnerioideae includes most of the New World members of the Gesneriaceae family and is currently considered to include five tribes: Beslerieae, Episcieae, Gesnerieae, Gloxinieae, and Napeantheae. This study presents maximum parsimony and maximum likelihood phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer regions (ITS), and the chloroplast DNA *trnL* intron, *trnL-trnF* intergenic spacer region, and *trnE-trnT* intergenic spacer region sequences. The ITS and cpDNA data sets strongly support the monophyly of a Beslerieae/Napeantheae clade; an Episcieae clade; a Gesnerieae clade; a Gloxinieae clade minus *Sinningia*, *Sinningia* relatives, and *Gloxinia sarmentiana*; and a *Sinningia*/*Paliavana*/*Vanhouttea* clade. This is the first study to provide strong statistical support for these tribes/clades. These analyses suggest that *Sinningia* and relatives should be considered as a separate tribe. Additionally, generic relationships are explored, including the apparent polyphyly of *Gloxinia*. Chromosome number changes are minimized on the proposed phylogeny, with the exception of the $n = 11$ taxa of the Gloxinieae. Scaly rhizomes appear to have been derived once in the Gloxinieae sensu stricto. The number of derivations of the inferior ovary is unclear: either there was one derivation with a reversal to a superior ovary in the Episcieae, or there were multiple independent derivations of the inferior ovary.

Key words: Gesneriaceae; Gesnerioideae; ITS; molecular phylogenetics; *trnE-T*; *trnL-F*.

The Gesneriaceae Dumort. are a moderate-sized tropical family of flowering plants comprising ~133 genera and >2500 species (Brummitt, 1992; Wentsai et al., 1998). This family is a member of the order Lamiales (sensu Olmstead et al., 1993; Angiosperm Phylogeny Group, 1998) and is distinguished from other members of the order by a suite of characters: five-lobed corollas, parietal placentation, unilocular bicarpellate ovaries, a pair-flowered cyme inflorescence, minute seeds, and presence of endosperm in the seeds of many taxa (Cronquist, 1981; Wiehler, 1983; Smith et al., 1997b). Order-wide phylogenetic studies have suggested that the Gesneriaceae form a separate lineage from the presumed nearest relatives, such as the Acanthaceae, Bignoniaceae, Scrophulariaceae, and Veronicaceae (Oxelman, Backlund, and Bremer, 1999; Olmstead et al., 2000, 2001).

[1] Manuscript received 26 April 2001; revision accepted 9 August 2001.

The authors thank Michael Bordelon and Leslie Brothers of the Smithsonian Botany Research Greenhouses for help with growing (and keeping alive) a diversity of Gesneriaceae; Charlie Fenster and Michele Dudash of the University of Maryland for stimulating collaboration between the Skog and Zimmer research groups and for helpful discussions in beginning this project; John L. Clark, Linda Prince, Warren Wagner, and Jim Wilgenbusch for reviewing earlier versions of the manuscript; Jim Wilgenbusch and David Swofford of the Smithsonian LMS for help with the maximum likelihood analyses; Ken Karol and Fiery Cushman for preliminary sequencing for this study; and the Smithsonian Institution Postdoctoral Fellowship in Molecular Evolution and the Smithsonian Institution Laboratory of Molecular Systematics for supporting this research.

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The number of species recognized in the Gesneriaceae and the superspecific classification of those species (e.g., generic circumscription) are matters about which there has been some disagreement (Kvist and Skog, 1993; Smith, 1994; Burtt and Wiehler, 1995). Overviews of the history of Gesneriaceae classification can be found in Wiehler (1983) and Smith et al. (1997b). Three subfamilies are currently recognized in the Gesneriaceae: Gesnerioideae Dumort., Cyrtandroideae Endl., and Coronantheroideae Wiehler (Burtt and Wiehler, 1995). The Gesnerioideae are separated from the Cyrtandroideae by having isocotylous seedling leaves rather than anisocotylous leaves, as well as being distributed in the New World rather than the Old World (Wiehler, 1983), and are separated from the Coronanthereae by having a nectary free from the ovary rather than embedded in the basal part of the ovary (Wiehler, 1983). The Gesnerioideae are currently divided into five tribes: Beslerieae Bartl. & H.L. Wendl., Episcieae Endl., Gesnerieae, Gloxinieae Fritsch, and Napeantheae Wiehler (Burtt and Wiehler, 1995). Morphological variation in New World Gesnerioideae is pronounced, particularly in terms of flower appearance, leading some to the suggestion that this wide variation in flower shape and color has played a large role in the confusion surrounding generic circumscription (Wiehler, 1983; Kvist and Skog, 1996). Indeed, some species have been considered as members of numerous genera (e.g., *Drymonia serrulata* (Jacq.) Mart. has been considered a member of *Alloplectus* Mart., *Besleria* L., *Columnea* L., and *Drymonia* Mart.).

Recent phylogenetic studies have explored relationships within and among the Gesnerioideae tribes using the chloroplast gene *ndhF*, the nuclear ribosomal DNA internal transcribed spacer region (ITS; Episcieae only), and morphological characters (Smith, 1996, 2000a, b, c; Smith et al., 1996,

1997a, b; Smith and Carroll, 1997; Smith and Atkinson, 1998). Lack of resolution and low statistical support (i.e., low bootstrap values) in these studies have prevented researchers from making a confident assessment of tribal and generic relationships. This study presents maximum parsimony and maximum likelihood phylogenetic analyses of nrDNA ITS region, and the chloroplast DNA *trnL* intron, *trnL-trnF* intergenic spacer, and *trnE-trnT* intergenic spacer region sequences. These analyses are used to address three primary questions: (1) Does the current classification of the Gesnerioideae proposed by Burtt and Wiehler (1995) reflect inferred phylogenetic relationships? (2) What are the generic relationships within each major clade/tribe? (3) Are morphological and cytological characteristics used to delineate these groups of the Gesnerioideae congruent with the phylogenetic hypotheses derived from the DNA sequence data?

MATERIALS AND METHODS

Sampling—Samples were selected from live plants grown at the Smithsonian's National Museum of Natural History Botany Research Greenhouses, Suitland, Maryland, USA, and one herbarium specimen at US (*Reldia minutiflora* [L.E. Skog] L.P. Kvist & L.E. Skog). Outgroup sampling was restricted to *Aeschynanthus* W.Jack and *Streptocarpus* Lindl. (subfamily Cyrtandroideae; Burtt and Wiehler, 1995; species and voucher information has been archived at the Botanical Society of America website at http://ajbsupp.botany.org/) because previous studies have supported the monophyly of the Gesnerioideae (Smith and Carroll, 1997; Smith and Atkinson, 1998). The ingroup comprised 57 species representing all five tribes of the Gesnerioideae, including 16 genera of tribe Gloxinieae, 12 genera of tribe Episcieae, 3 genera of tribe Beslerieae, the 1 genus of tribe Napeantheae, and 2 genera of tribe Gesnerieae (http://ajbsupp.botany.org/). Some greenhouse plants included in this study died before being vouchered (marked ''not vouchered'' in the table at http://ajbsupp.botany.org/), but their identity was verified by L. E. Skog. Some of these unvouchered taxa are *Gesneria* L. and *Rhytidophyllum* Mart. specimens obtained from the Montreal Botanical Garden, Montreal, Quebec, Canada. Whether they have been vouchered there is unknown. The sample labeled *Rhytidophyllum vernicosum* Urb. & Ekman may not be that species but a closely related undescribed species. Until additional samples of this collection can be obtained, its exact disposition cannot be verified. Regardless, in the molecular trees, the phylogenetic position of the sample identified as *R. vernicosum* is where either *R. vernicosum* or the undescribed species of *Rhytidophyllum* would be expected to occur.

DNA sequencing—DNA was isolated using standard CTAB (hexadecyltrimethylammonium bromide) extraction methods (Doyle and Doyle, 1987) or the Qiagen DNeasy DNA isolation kit (Qiagen, Valencia, California, USA). Templates of the nrDNA ITS region were prepared using the primers ITS5HP (5′-GGA AGG AGA AGT CGT AAC AAG G-3′; Suh et al., 1993) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′; White et al., 1990). The chloroplast spacer regions were amplified using the primers trnLc (5′-CGA AAT CGG TAG ACG CTA CG-3′) and trnLf (5′-ATT TGA ACT GGT GAC ACG AG-3′) for the *trnL* intron and *trnL-trnF* intergenic spacer (igs; Taberlet et al., 1991), respectively, and trnE (5′- GCC TCC TTG AAA GAG AGA TG-3′) and trnTr (5′- TAC CAC TGA GTT AAA AGG GC -3′) for the *trnE-trnT* igs (Doyle et al., 1992). Polymerase chain reaction (PCR) amplifications followed the procedures described by Baldwin (1992), Baldwin et al. (1995), and Roalson and Friar (2000) utilizing Taq DNA polymerase (Promega, Madison, Wisconsin, USA). Mg HotBeads (3.0 mM; Lumitekk, Salt Lake City, Utah, USA) were used with recalcitrant templates. The PCR products were electrophoresed in a 1.0% agarose gel in 1× TBE (pH 8.3) buffer, stained with ethidium bromide to confirm a single product, and purified using the PEG (polyethylene glycol 8000) precipitation procedure (Johnson and Soltis, 1995). Direct cycle sequencing of purified template DNAs followed the manufacturer's specifications, using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, California, USA) and the PCR primers. Sequencing was performed using an Applied Biosystems Model 377 Automated DNA Sequencing System (PE Biosystems).

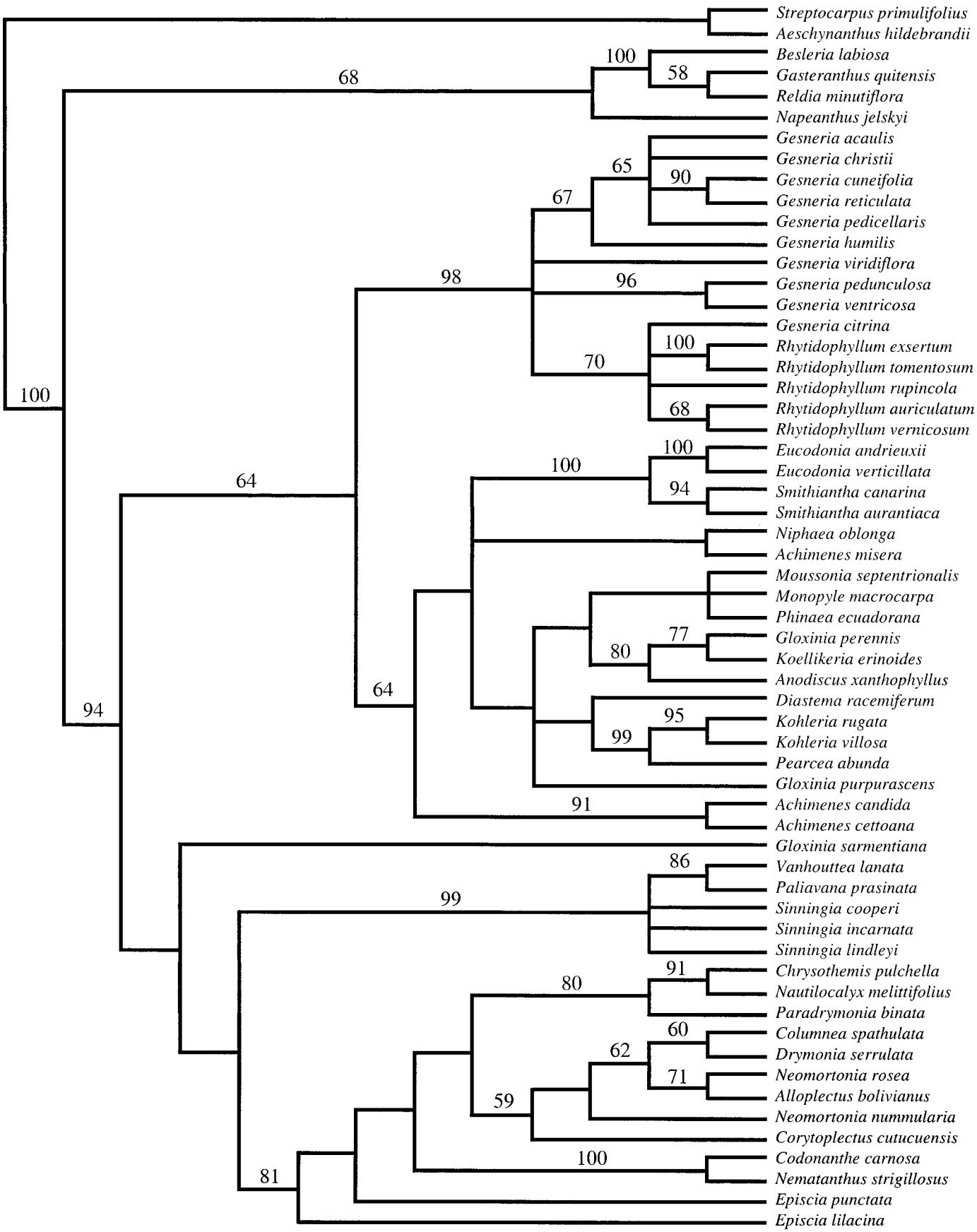
DNA chromatograms were proofed, edited, and chromatograms were assembled using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA). The sequences were truncated to include only ITS1, 5.8S, ITS2, the *trnL* intron, the *trnL-trnF* igs, and the *trnE-trnT* igs. Identification of the ends of ITS1 and ITS2 were based on comparisons with sequences from other GenBank sequences (*Harveya squamosa* [Orobanchaceae] GBAN-AF120225; *Whytockia bijieensis* [Gesneriaceae] GBAN-AF055058). (The prefix GBAN- has been added to the accession numbers to link the online version of *American Journal of Botany* with GenBank but is not part of the actual accession number.) The ends of the chloroplast spacers were determined by comparisons with other chloroplast spacer sequences (Taberlet et al., 1991; *Nicotiana tabacum* [chloroplast complete genome; Solanaceae] GBAN-NC001879). All sequences were manually aligned.

Sequences have been deposited in GenBank (ITS accessions GBAN-AY047039 to GBAN-AY047097; *trnL-F* accessions GBAN-AY047098 to GBAN-AY047156; *trnE-T* accessions GBAN-AY047157 to GBAN-AY047215; *http://ajbsupp.botany.org/v89*).

Phylogenetic analyses—Maximum parsimony (MP) analyses were performed using PAUP*4.0b4a (Swofford, 2001). The analysis used heuristic searches (ACCTRAN; 100 RANDOM ADDITION cycles; tree bisection-reconstruction (TBR) branch swapping; STEEPEST DESCENT). The MP analysis of cpDNA spacers was limited to 200 trees of equal length for each of the 100 replicates due to the large number of equal-length trees. Clade robustness was estimated using the parsimony jackknife (jk) analysis (10 000 ''fast addition'' heuristic replicates, ''jac'' emulated, 33% deletion; Farris et al., 1996; Mort et al., 2000). The fast addition heuristic search is a method in which each replicate is performed using one random-sequence-addition replicate and no branch swapping. Very similar values were found using 10 000 fast addition bootstrap replicates (data not shown). Three ingroup data sets were analyzed and compared: ITS, *trnL-F* + *trnE-T*, and a combination of these. Some gaps (indels) in the data matrices were mapped onto the combined analysis strict consensus tree (Fig. 3). Autapomorphic gaps and complex gaps (such as those associated with single-base repeat regions) were not included as mapped characters.

Homogeneity of the ITS and *trnL-F* + *trnE-T* data sets was assessed using three tests: the partition homogeneity test (Farris et al., 1995) as implemented in PAUP*4.0b4a, assessment of branch support conflict (de Queiroz, Donoghue, and Kim, 1995), and the likelihood ratio test as implemented by Modeltest 2.1 (Posada and Crandall, 1998). The cpDNA spacers were automatically combined, as they are both part of the nonrecombining chloroplast and any differences between them are likely due to homoplasy rather than separate histories. With the partition homogeneity test, 10 000 replicate data partitions were run (heuristic search; simple addition; no branch swapping), excluding constant characters. This test measures character congruence by comparing tree-length differences among trees derived from resampled data partitions of the combined data sets and trees derived from the defined data partition (i.e., nrDNA vs. cpDNA). Differences in branch support between the different data sets were assessed by comparing jackknife support values for branches in conflict. Conflicting branch structure that was well supported (jk $>$ 67%) as being different between the ITS and cpDNA data sets was considered to be in conflict, while branch structure that was different, but not well supported by one or both data sets, was not considered to represent conflict. The ITS and cpDNA data sets were analyzed in separate maximum likelihood (ML) analyses based on the likelihood ratio test as implemented by Modeltest 2.1 (Posada and Crandall, 1998).

The nrDNA ITS and cpDNA *trnL-F*/*trnE-T* spacer regions were analyzed separately with ML as implemented in PAUP*4.0b4a (Swofford, 2001). Heuristic searches were employed (ACCTRAN; starting tree based on neighbor-joining reconstruction; TBR branch swapping; STEEPEST DESCENT). The general time reversable (GTR) model of evolution (Yang, 1994a) with an estimated gamma shape parameter (gamma) and estimated proportion of invariant sites (p-inv) was used in the ML analysis of ITS (Gu, Fu, and Li,


Streptocarpus primulifolius
Aeschynanthus hildebrandii
Besleria labiosa
Gasteranthus quitensis
Reldia minutiflora
Napeanthus jelskyi
Gesneria acaulis
Gesneria christii
Gesneria cuneifolia
Gesneria reticulata
Gesneria pedicellaris
Gesneria humilis
Gesneria viridiflora
Gesneria pedunculosa
Gesneria ventricosa
Gesneria citrina
Rhytidophyllum exsertum
Rhytidophyllum tomentosum
Rhytidophyllum rupincola
Rhytidophyllum auriculatum
Rhytidophyllum vernicosum
Eucodonia andrieuxii
Eucodonia verticillata
Smithiantha canarina
Smithiantha aurantiaca
Niphaea oblonga
Achimenes misera
Moussonia septentrionalis
Monopyle macrocarpa
Phinaea ecuadorana
Gloxinia perennis
Koellikeria erinoides
Anodiscus xanthophyllus
Diastema racemiferum
Kohleria rugata
Kohleria villosa
Pearcea abunda
Gloxinia purpurascens
Achimenes candida
Achimenes cettoana
Gloxinia sarmentiana
Vanhouttea lanata
Paliavana prasinata
Sinningia cooperi
Sinningia incarnata
Sinningia lindleyi
Chrysothemis pulchella
Nautilocalyx melittifolius
Paradrymonia binata
Columnea spathulata
Drymonia serrulata
Neomortonia rosea
Alloplectus bolivianus
Neomortonia nummularia
Corytoplectus cutucuensis
Codonanthe carnosa
Nematanthus strigillosus
Episcia punctata
Episcia lilacina
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1995). Gamma and p-inv were used to measure among-site rate variation. The Kimura three parameter with unequal base frequencies (K3Puf) model of evolution (Kimura, 1981) with an estimated gamma shape parameter (Yang, 1994b) was used on the ML analysis of cpDNA spacer data. The choice of these models for the separate data sets was based on the results of analyses using Modeltest 2.1 (Posada and Crandall, 1998). The Modeltest 2.1 analysis tests the fit of various ML models to the data set and estimates base change frequencies, proportion of variable characters, and shape of the gamma distribution, and chooses the model that best fits the data using the hierarchical likelihood ratio test (Posada and Crandall, 1998).

Morphological characters and chromosome numbers—Morphological characters mapped onto the phylogenetic trees were based on examination of live and herbarium specimens as well as reports from the literature (e.g., Wiehler, 1983) using the program MacClade (Sinauer Associates, Sunderland, Massachusetts, USA). Characters were unordered and mapped using the minimal change option. Chromosome numbers were taken from the literature (Skog, 1984; Kvist and Skog, 1996). In the case of species included in the analyses that have not been previously counted, they were assumed to have the chromosome number of other members of the genus. While this assumption could have misled inferences of chromosome evolution, we did not expect it to impact this study to any large degree due to the relative rarity of changes in chromosome number across the Gesnerioideae (Skog, 1984).

RESULTS

DNA sequencing and alignment—The two ITS sequencing primers produced overlapping fragments that collectively covered the entire spacer and 5.8S rDNA regions along both strands. The aligned ITS data matrix was 731 base pairs (bp) long with 424 variable sites, of which 322 were parsimony informative. The length of the unaligned sequences varied from 608 to 645 bp. Three sequences are missing a portion (52–81 aligned bp) of the 5′ end of the ITS1 spacer due to poor sequencing of that region (*Paliavana prasina* (Ker Gawl.) Fritsch, *Pearcea abunda* (wiehler) L.P. Kvist & L.E. Skog, and *Vanhouttea lanata* Fritsch). The alignment of the ITS region was somewhat difficult, particularly between the outgroups, members of the Beslerieae and Napeantheae, and the rest of the subfamily. Multiple alignments were explored (data not shown) without major changes to tree topology. Two regions of the nrDNA spacers (a 53-bp region of ITS1, exclusion set 1, and a 66-bp region of ITS2, exclusion set 2) were particularly difficult to align with confidence. These regions were excluded separately and collectively in additional analyses (data not shown). These alternative analyses resulted in no major topological rearrangements to well-supported branches (branches with >67% jk), but did lower support for some clades. Exclusion set 1 resulted in a nearly identical support tree. Exclusion set 2 and the combined exclusion of sets 1 and 2 resulted in the drop of statistical support of the association of *Napeanthus* with the Beslerieae clade to below 50% and reduced the statistical support for the pairing of the Gesnerieae clade and the Gloxinieae clade to <50%. The most inclusive alignment resulted in 42 gaps ranging from 1 to 22 bp in length. Eleven of these gaps were single-base indels. This data alignment resulted in uncorrected pairwise sequence divergence of 0–34%.

The two *trnL-F* sequencing primers produced overlapping fragments that collectively covered the entire *trnL* intron, *trnL* exon 2, and the *trnL-F* intergenic spacer along both strands. The two *trnE-T* sequencing primers produced overlapping fragments that collectively covered the entire *trnE-T* intergenic spacer along both strands except for 20–40 bp of the 3′ end of the spacer. The aligned *trnL-F/trnE-T* data matrix was 1928 bp long with 496 variable sites, of which 210 were parsimony informative. The length of the unaligned sequences varied from 681 to 907 bp for the *trnL-F* spacers and 388 to 836 bp for the *trnE-T* spacer. Twenty sequences are missing a portion (9–46 bp) of the 5′ end of the *trnL* intron and one sequence is missing 18 bp of the 3′ end of the *trnL-F* igs due to poor sequencing of that region. The alignment of the *trnL-F*/*trnE-T* region was, for the most part, unambiguous. One region of the cpDNA spacers (3′ end of the *trnE-T* igs, 58 bp long) was somewhat difficult to align confidently. This region is a complex microsatellite-like repeat and was excluded in additional analyses (data not shown). The alternative analysis resulted in only one change in statistically well-supported branches. The branch grouping all of the Episcieae tribe was reduced to <50% support. The most inclusive alignment resulted in 71 gaps ranging from 1 to 467 bp in length (34 gaps of 1–200 bp in the *trnL-F* spacer region and 37 gaps of 1–467 bp in the *trnE-T* spacer region). Seventeen of these gaps were single-base indels. This data alignment resulted in uncorrected pairwise sequence divergence of 0–9%.

Maximum parsimony analysis—Maximum parsimony analysis of the ITS Gesnerioideae data set resulted in 5400 most-parsimonious trees (length = 1698 steps, consistency index [CI] = 0.443, retention index [RI] = 0.637, rescaled consistency index [RC] = 0.282). Figure 1 is the strict consensus of these trees. Maximum parsimony analysis of the *trnL-F*/*trnE-T* Gesnerioideae data set resulted in 18 400 most-parsimonious trees (length = 719 steps, CI = 0.779, RI = 0.822, RC = 0.640). Figure 2 is the strict consensus of these trees. Maximum parsimony analysis of the combined ITS/*trnL-F*/*trnE-T* Gesnerioideae data set resulted in 12 most-parsimonious trees (length = 2452 steps, CI = 0.534, RI = 0.674, RC = 0.361). Figure 3 is the strict consensus of these trees.

Tests of conflict between data sets—The partition homogeneity test found a significant difference between the nrDNA/cpDNA partition and random partitioning ($P = 0.0004$). Similarly, the likelihood ratio test found that the fit of separate models to the ITS and cpDNA data sets was significantly better ($P < 0.01$) than a model combining the data sets (data not shown).

Generally, the ITS and cpDNA individual analyses are congruent, with some slight differences among poorly supported nodes (Figs. 1 and 2). Two sets of moderately supported nodes are in conflict. In the ITS MP analysis, *Gloxinia perennis* (L.) Fritsch and *Koellikeria erinoides* (DC.) Mansf. are moderately supported as sister taxa (jk = 77%). In the cpDNA MP analysis, *Koellikeria* Regel is paired with *Gloxinia purpurascens* (Rusby) Wiehler (jk = 65%; Figs. 1 and 2). Additionally, the

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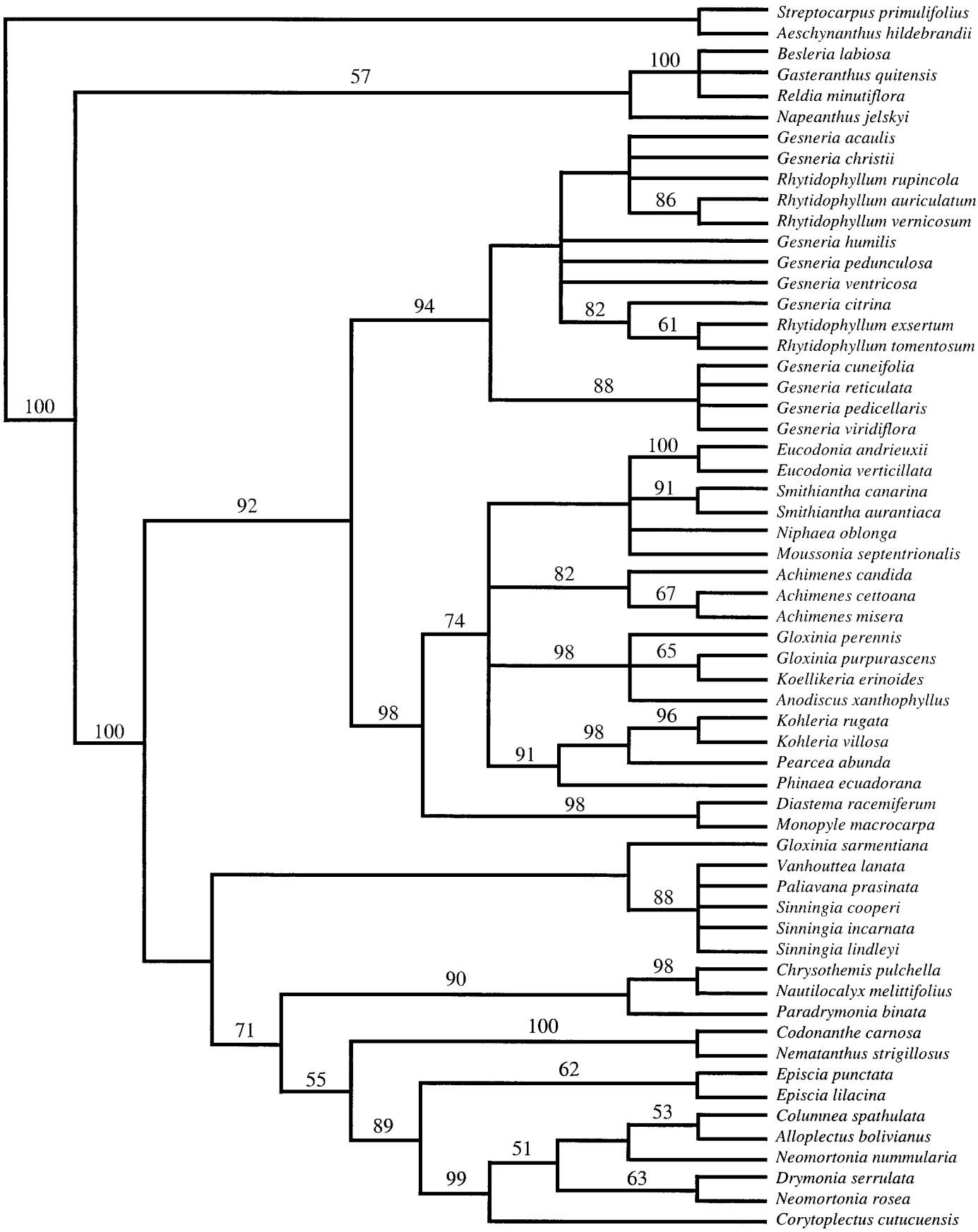
Fig. 1. Analysis of relationships within Gesnerioideae using the nrDNA ITS data set. Strict maximum parsimony (MP) consensus tree of 5400 most-parsimonious trees of 1698 steps consistency index [CI] = 0.443, retention index [RI] = 0.637, rescaled consistency index [RC] = 0.282). Numbers above branches are jackknife percentages where branch support is >50%.


Streptocarpus primulifolius
Aeschynanthus hildebrandii
Besleria labiosa
Gasteranthus quitensis
Reldia minutiflora
Napeanthus jelskyi
Gesneria acaulis
Gesneria christii
Rhytidophyllum rupincola
Rhytidophyllum auriculatum
Rhytidophyllum vernicosum
Gesneria humilis
Gesneria pedunculosa
Gesneria ventricosa
Gesneria citrina
Rhytidophyllum exsertum
Rhytidophyllum tomentosum
Gesneria cuneifolia
Gesneria reticulata
Gesneria pedicellaris
Gesneria viridiflora
Eucodonia andrieuxii
Eucodonia verticillata
Smithiantha canarina
Smithiantha aurantiaca
Niphaea oblonga
Moussonia septentrionalis
Achimenes candida
Achimenes cettoana
Achimenes misera
Gloxinia perennis
Gloxinia purpurascens
Koellikeria erinoides
Anodiscus xanthophyllus
Kohleria rugata
Kohleria villosa
Pearcea abunda
Phinaea ecuadorana
Diastema racemiferum
Monopyle macrocarpa
Gloxinia sarmentiana
Vanhouttea lanata
Paliavana prasinata
Sinningia cooperi
Sinningia incarnata
Sinningia lindleyi
Chrysothemis pulchella
Nautilocalyx melittifolius
Paradrymonia binata
Codonanthe carnosa
Nematanthus strigillosus
Episcia punctata
Episcia lilacina
Columnea spathulata
Alloplectus bolivianus
Neomortonia nummularia
Drymonia serrulata
Neomortonia rosea
Corytoplectus cutucuensis
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cpDNA analysis supports a polytomy including *Gesneria cuneifolia* (DC.) Fritsch, *G. pedicellaris* Alain, *G. reticulata* (Griseb.) Urb., and *G. viridiflora* (Decne.) Kuntze (jk = 88%), while in the ITS parsimony analysis, *G. cuneifolia* and *G. reticulata* form a species pair (jk = 90%) within a clade including *G. acaulis* L., *G. christii* Urb., and *G. pedicellaris* (jk = 65%), sister to *G. humilis* L. (jk = 67%), with *G. viridiflora* in a unresolved polytomy outside of this clade (Figs. 1 and 2).

There is as yet no consensus as to when data sets should be combined (reviewed in de Queiroz, Donoghue, and Kim, 1995). While the partition homogeneity test provides a test of congruence among data sets, it is not clear how the test is affected by differences in gene history vs. homoplasy (Miller, Rausher, and Manos, 1999). The likelihood ratio test explores differences in the model of nucleotide change, not necessarily topological differences supported by the data sets. Additionally, many authors consider simultaneous analysis of all data to be the most effective way to study evolutionary descent (Thornton and DeSalle [2000] and references therein). In this study, the incongruences between the data sets do not generally involve well-supported branches. In fact, the relationships that this study is most interested in addressing (tribal relationships) have the same topology in the separate analyses. Therefore, the data sets were combined to optimize the resolving power of all of the data in a single analysis.

The combined data parsimony analysis trees are a hybrid between the ITS and cpDNA topologies (Figs. 1–3). Strongly supported nodes based on only one data set are generally present and strongly supported in the combined analysis (e.g., the *Gloxinia* L'Heritier/*Anodiscus* Benth./*Koellikeria* clade; Figs. 1–3). There are some cases where clades strongly supported by one data set have less support in the combined analysis than in either of the separate analyses, but these are infrequent (e.g., the *Diastema* Benth./*Monopyle* Benth. clade; Figs. 1–3). Generally, the combined analysis represents the strongly supported nodes of the individual analyses.

Maximum likelihood analysis—The ML analyses used the parameters listed in Table 1. The ML analysis of the ITS data set examined 52 475 rearrangements. One tree ($-\ln$ = 8906.53324) was found (Fig. 4). The ML analysis of the *trnL-F*/*trnE-T* data set examined 46 151 rearrangements. One tree ($-\ln$ = 7402.75877) was found (Fig. 5). Differences between the ML analyses were largely the same as the differences between MP analyses of the two data sets.

Comparison of MP and ML trees—The MP and ML analyses of the individual data sets are generally congruent, with slight differences in branching topology among poorly supported (MP) nodes (jk < 50%). In the ITS analyses, the ML analysis differs from the MP analysis in its placement of *Achimenes* Pers. and *Niphaea* Lindl., the placement of *Diastema*, and the pattern by which the major clades in the Episcieae tribe group together (Figs. 1 and 4). In the cpDNA analyses, the ML analysis and the MP analysis are completely congruent, although some branches in the ML analysis are unresolved in the MP strict consensus (Figs. 2 and 5).

DISCUSSION

Congruence of previous classification to phylogenetic hypotheses—The combined analysis closely resembles the tribal classification of Burtt and Wiehler (1995), which was based on morphology, chromosome numbers, and geographic distribution, with the exception of the placement of *Sinningia* Nees and its relatives and of *Gloxinia sarmentiana* Gardner ex Hook. (Figs. 3 and 6). The exclusion of *Sinningia*, *Vanhouttea* Lemaire, and *Paliavana* Vandelli from the Gloxinieae to form a new tribe has been suggested previously (Smith and Carroll, 1997; Smith et al., 1997b; Smith and Atkinson, 1998). While these earlier studies did not demonstrate statistical support for this branching topology, the analyses presented here do (Figs. 1–3). The placement of *Gloxinia sarmentiana* outside of the Gloxinieae tribe is a novel finding and is discussed in detail below in the section regarding the *Sinningia* clade and *Gloxinia sarmentiana*.

The phylogenetic hypotheses presented here give strong statistical support for the major lineages of the Gesnerioideae. As previously suggested (Smith, 2000a), the Napeantheae is associated with the Beslerieae (jk = 76%), and the Beslerieae form a monophyletic group (jk = 100%; Figs. 3 and 6). This Napeantheae/Beslerieae clade is strongly supported as sister to the rest of the Gesnerioideae (jk = 100%). The *Sinningia* and relatives clade is strongly supported (jk = 100%; Figs. 3 and 6). Given the phylogenetic hypotheses presented here and the strong support for the groupings, the reinstatement of the Sinningieae of Fritsch (1893–1894), with the additional inclusion of *Vanhouttea* and *Paliavana* in this tribe, seems warranted.

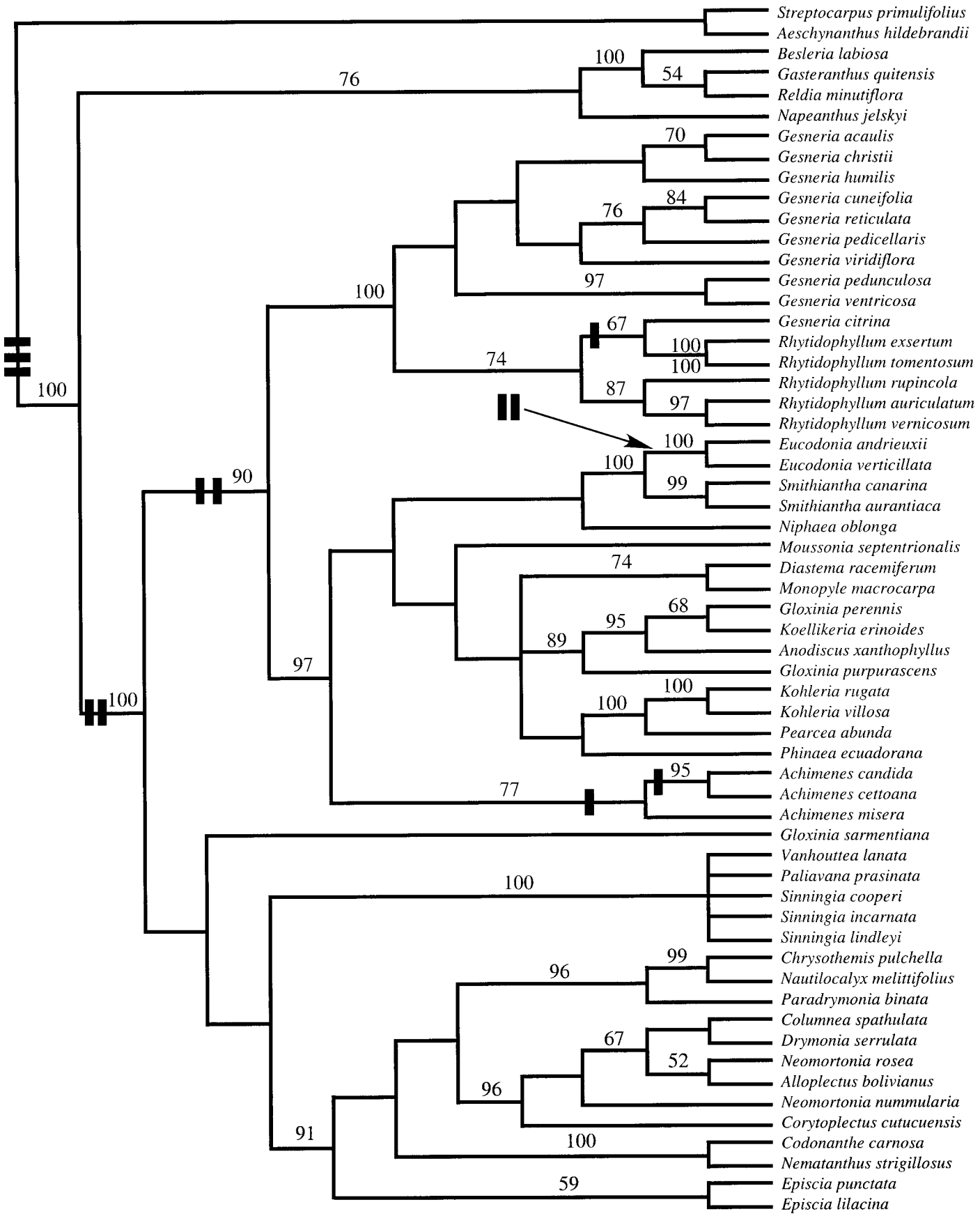
The three other tribes are all strongly supported as monophyletic. The Gloxinieae and Gesnerieae tribes form a sister pair (jk = 90%; Figs. 3 and 6) with strong support for the monophyly of each tribe (Gloxinieae: jk = 97%; Gesnerieae: jk = 100%; Figs. 3 and 6). How the Episcieae is related to these other groups is not entirely clear as it is weakly supported as sister to the *Sinningia* lineage (jk < 50%), but the tribe is well supported as monophyletic (jk = 91%; Figs. 3 and 6).

Relationships within the Beslerieae—Only three genera of the Beslerieae are sampled here. The branches grouping these genera are not well supported, but the topology they suggest requires comment. The relationships suggested here place *Gasteranthus* Benth. as sister to *Reldia* Wiehler, and this pair sister to *Besleria* (Fig. 3). *Gasteranthus* often has been considered to be congeneric with *Besleria* (see discussion in Skog and Kvist, 2000). Previous phylogenetic studies have placed *Besleria* and *Gasteranthus* as sister taxa (Smith, 2000a), but with poor statistical support (bootstrap = 44%). Interestingly, *Reldia* and *Gasteranthus* share some morphological features including clusters of stomata (Skog and Kvist, 2000; Smith, 2000a). More detailed studies will be required to explore relationships among these genera.

Relationships within the Episcieae—Previous studies have not provided statistical support for how the genera/lineages of

←

Fig. 2. Analysis of relationships within Gesnerioideae using the cpDNA *trnL-F*/*trnE-T* data set. Strict (MP) consensus tree of 18 400 most-parsimonious trees of 719 steps (CI = 0.779, RI = 0.822, RC = 0.640). Numbers above branches are jackknife percentages where branch support is >50%.


Streptocarpus primulifolius
Aeschynanthus hildebrandii
Besleria labiosa
Gasteranthus quitensis
Reldia minutiflora
Napeanthus jelskyi
Gesneria acaulis
Gesneria christii
Gesneria humilis
Gesneria cuneifolia
Gesneria reticulata
Gesneria pedicellaris
Gesneria viridiflora
Gesneria pedunculosa
Gesneria ventricosa
Gesneria citrina
Rhytidophyllum exsertum
Rhytidophyllum tomentosum
Rhytidophyllum rupincola
Rhytidophyllum auriculatum
Rhytidophyllum vernicosum
Eucodonia andrieuxii
Eucodonia verticillata
Smithiantha canarina
Smithiantha aurantiaca
Niphaea oblonga
Moussonia septentrionalis
Diastema racemiferum
Monopyle macrocarpa
Gloxinia perennis
Koellikeria erinoides
Anodiscus xanthophyllus
Gloxinia purpurascens
Kohleria rugata
Kohleria villosa
Pearcea abunda
Phinaea ecuadorana
Achimenes candida
Achimenes cettoana
Achimenes misera
Gloxinia sarmentiana
Vanhouttea lanata
Paliavana prasinata
Sinningia cooperi
Sinningia incarnata
Sinningia lindleyi
Chrysothemis pulchella
Nautilocalyx melittifolius
Paradrymonia binata
Columnea spathulata
Drymonia serrulata
Neomortonia rosea
Alloplectus bolivianus
Neomortonia nummularia
Corytoplectus cutucuensis
Codonanthe carnosa
Nematanthus strigillosus
Episcia punctata
Episcia lilacina
100
76
100
54
70
84
76
97
100
67
74
100
100
87
97
100
90
100
99
74
68
95
89
97
100
100
100
95
77
100
96
99
67
52
96
91
100
59

the Episcieae are related (Smith et al., 1997b; Smith and Carroll, 1997; Smith, 2000b). Thirteen species in 11 genera of the Episcieae were included in this study. Several relationships are well supported by the data, including the sister relationship of *Codonanthe* (Mart.) Hanst. and *Nematanthus* Schr. (jk = 100%), the close relationship of the species of *Chrysothemis* Decne., *Nautilocalyx* Hanst., and *Paradrymonia* Hanst. sampled (jk = 96%), and the grouping of *Alloplectus*, *Columnea*, *Corytoplectus* Oerst., *Drymonia*, and *Neomortonia* Wiehler (jk = 96%; Fig. 3). How these three clades are related to each other and to the weakly supported *Episcia* Mart. clade is not clear, and more detailed studies of the tribe will be necessary to explore these relationships.

Traditional taxonomic treatments have considered *Codonanthe* and *Nematanthus* as closely related (Chautems, 1984, 1988). These genera share an $n = 8$ chromosome complement, overlap in distribution in southern Brazil, and have been successfully crossed to produce fertile hybrids (Wiehler, 1977). Previous phylogenetic studies have either suggested that these genera were not sister taxa (Smith and Carroll, 1997) or that their grouping was weakly supported with the inclusion of *Codonanthopsis* Mansf. ($n = 9$; Smith et al., 1997b; Smith, 2000b). While the placement of *Codonanthopsis* cannot be addressed here, there is strong support for *Codonanthe* and *Nematanthus* being closely related.

Chrysothemis, *Nautilocalyx*, and *Paradrymonia* form a strongly supported clade in this study (Fig. 3). These three genera are quite diverse morphologically and include approximately 160 species. Previous studies have either placed *Chrysothemis* and *Nautilocalyx* together and *Paradrymonia* as unresolved in relation to this clade (Smith, 2000b) or a portion of *Paradrymonia* was weakly grouped with a *Chrysothemis*/*Nautilocalyx* clade (Smith and Carroll, 1997) using different species of these genera than those included here. *Chrysothemis* and *Nautilocalyx* both include species that are usually tuberous and tall stemmed, but *Paradrymonia* includes nontuberous and often short-stemmed plants. Given the morphological diversity of this group and the possible polyphyly of *Paradrymonia* (Smith and Carroll, 1997), generic circumscription and the relationships of all members of these genera to the rest of the Episcieae cannot be explicitly addressed here.

The genera *Alloplectus*, *Columnea*, *Corytoplectus*, *Drymonia*, and *Neomortonia* form a strongly supported clade (Fig. 3), unlike previous studies that have only provided weak support for this grouping (Smith and Carroll, 1997; Smith, 2000b). Additionally, one study suggested that *Neomortonia nummularia* (Hanst.) Wiehler was more closely related to *Episcia* (Smith and Carroll, 1997), a finding not supported by our data. The para-/polyphyly of *Neomortonia* is weakly supported in these analyses as in previous studies (Smith and Carroll, 1997; Smith, 2000b), but further study with greater resolution/sampling will be necessary to determine generic boundaries. Three of the genera of this clade have a berry fruit (*Columnea*, *Corytoplectus*, and *Neomortonia*), which is uncommon in the Gesnerioideae; the only other genera of Gesnerioideae included in this study with this character are *Codonanthe* and *Besleria*. *Alloplectus* and *Drymonia* both have the more common fleshy capsular fruit found in *Chrysothemis*, *Episcia*, *Nautilocalyx*, *Nematanthus*, and *Paradrymonia*.

TABLE 1. Parameters assigned to data sets for maximum likelihood analyses. Abbreviations are as follows: EBF = estimated base frequencies and SRM = substitution rate matrix. Proportion of invariable sites is not included under *trnL-F/trnE-T* as it is not a variable in the model used for this data set.

Parameters	ITS	*trnL-F/trnE-T*
EBF-A	0.230	0.346
EBF-C	0.260	0.168
EBF-G	0.253	0.163
EBF-T	0.257	0.323
Number of substitution types	6	3
Proportion of invariable sites	0.2588	N/A
Gamma distribution shape parameter	1.1731	0.6885
SRM-A/C	1.0122	1.0000
SRM-A/G	2.2666	1.5789
SRM-A/T	1.1819	0.3770
SRM-C/G	0.5184	0.3770
SRM-C/T	4.3128	1.5789
SRM-G/T	1.0000	1.0000

Episcia is represented here by two species, one (*E. lilacina* Hanst.) a member of *Episcia* sensu stricto and the other (*E. punctata* [Lindl.] Hanst.) a member of the segregate genus *Alsobia* Hanst. These species are only weakly supported as forming a clade (jk = 59%), and previous phylogenetic studies have not suggested a close relationship of these two (Smith and Carroll, 1997; Smith, 2000b). *Episcia* and *Alsobia* share a stoloniferous habit and sympodial shoot pattern that other segregates of *Episcia* (*Nautilocalyx* and *Paradrymonia*) do not have (Wiehler, 1983). More detailed studies are necessary to explore relationships among the elements of *Episcia*.

Relationships within the Gesnerieae—Fifteen species in two of the three genera in the Gesnerieae were included in this study (the third genus, the monotypic *Pheidonocarpa* L.E. Skog from Cuba and Jamaica, was not sampled). Despite previous suggestions that *Sanango* G.S. Bunting & J.A. Duke is nested within Gesnerioideae, tribe Gesnerieae (Dickison, 1994; Jensen, 1994; Norman, 1994; Wiehler, 1994; Burtt and Wiehler, 1995; Smith et al., 1997a), the genus now appears to be a lineage outside of Gesnerioideae and possibly the Gesneriaceae, along with the genus *Peltanthera* Benth. (Oxelman, Backlund, and Bremer, 1999; M. Kiehn, Institut für Botanik und Botanischer Garten, personal communication; E. H. Roalson and A. Idnurm, unpublished data). For these reasons, it was excluded from this study.

While the Gesnerieae is strongly supported as monophyletic, how the two included genera (*Gesneria* and *Rhytidophyllum*) are related is unclear, as the separate analyses suggest different topologies. In the sampling of five species, it appears that *Rhytidophyllum* is paraphyletic, with *Gesneria citrina* Urb. nested within *Rhytidophyllum* (Fig. 3). Three species groups are moderately to strongly supported in the combined data analysis. Group 1 comprises *G. cuneifolia* + *G. pedicellaris* + *G. reticulata* (jk = 76%), Group 2 comprises *G. pe-*

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Fig. 3. Analysis of relationships within Gesnerioideae using the combined nrDNA ITS and cpDNA *trnL-F*/*trnE-T* data sets. Strict (MP) consensus tree of 12 most-parsimonious trees of 2452 steps (CI = 0.534, RI = 0.674, RC = 0.361). Numbers above branches are jackknife percentages where branch support is >50%. Bars on branches refer to gaps supporting clades.

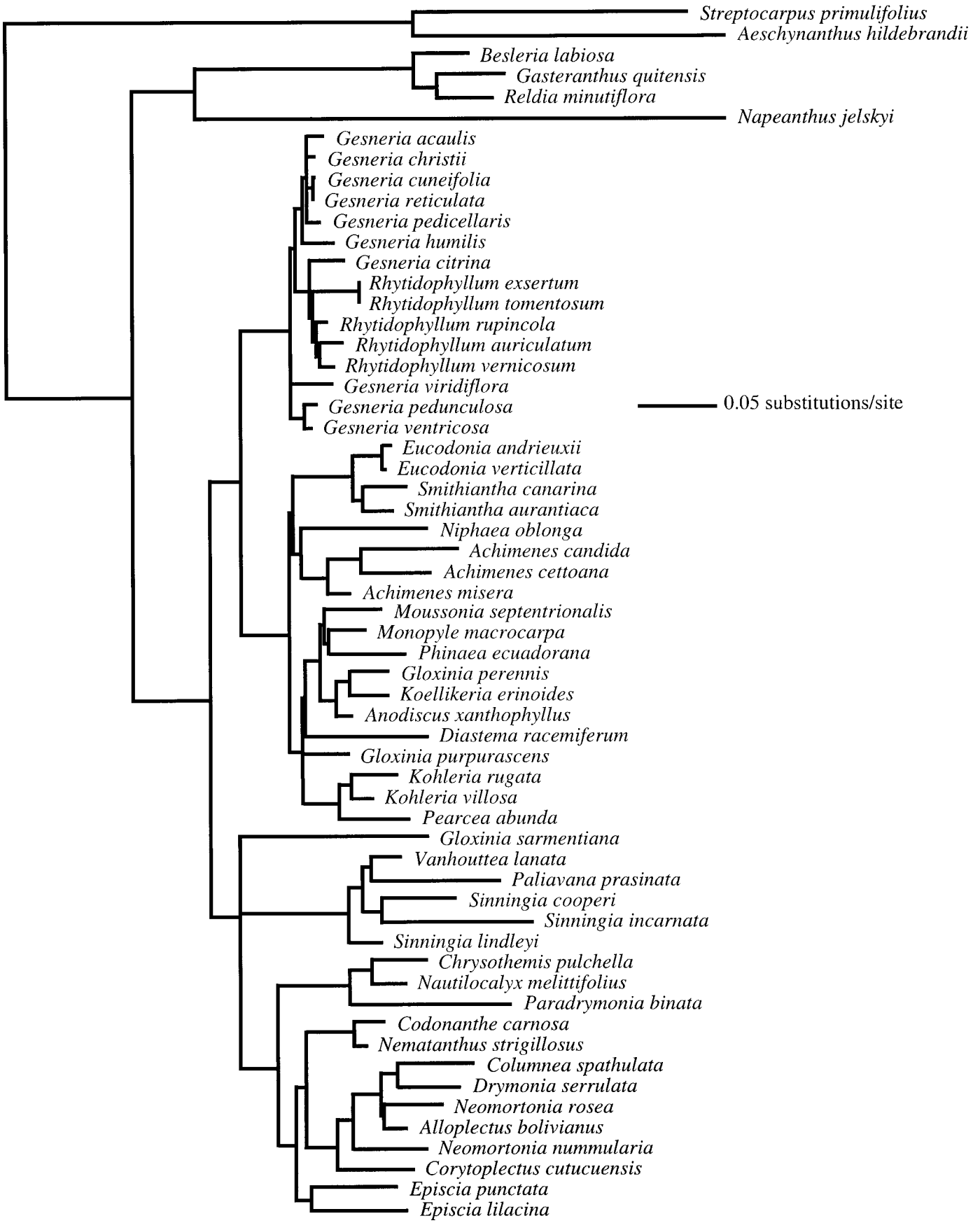


Fig. 4. Gesnerioideae internal transcribed spacer maximum likelihood tree (−ln = 8906.53324). Details of model parameters are listed in Table 1.

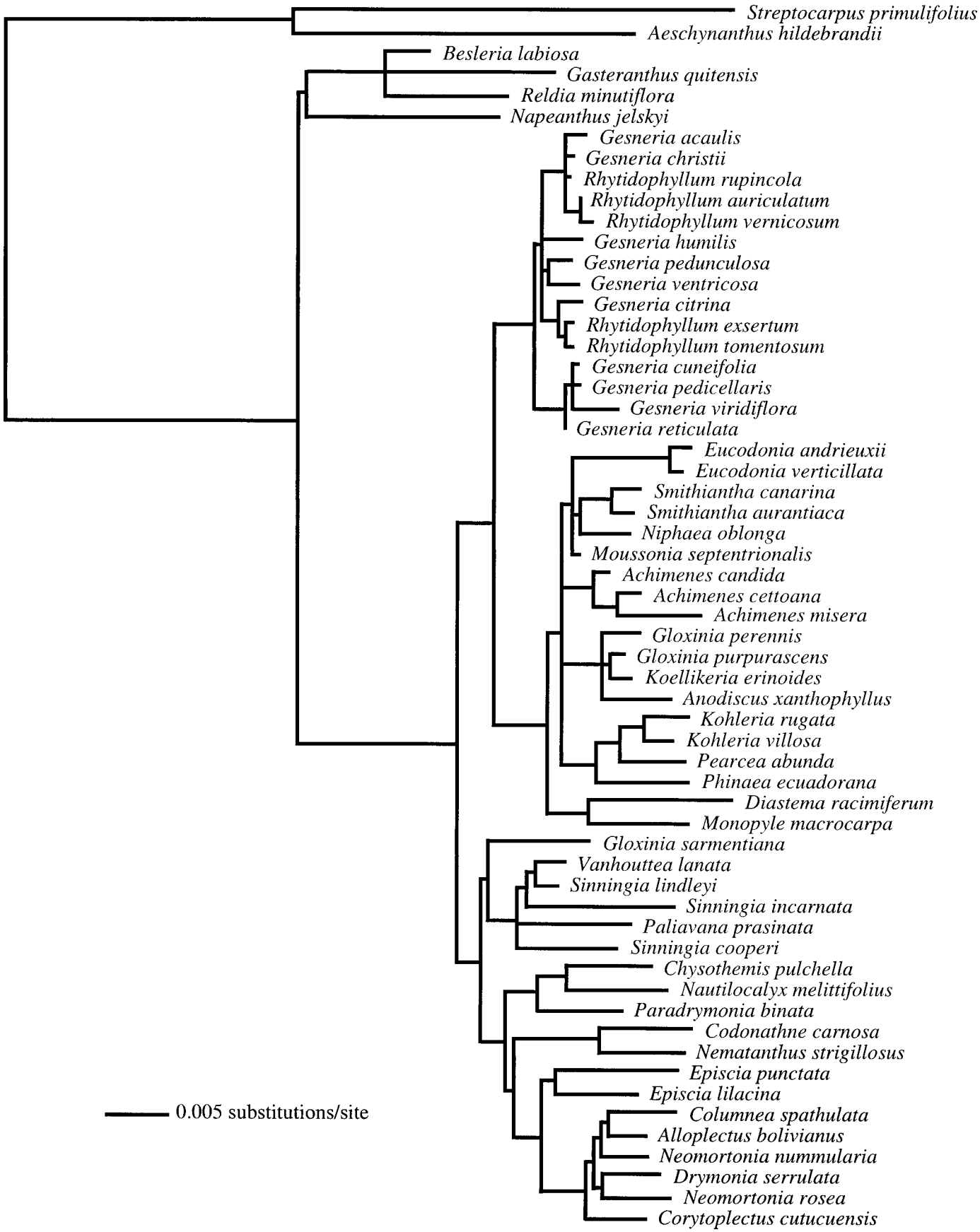
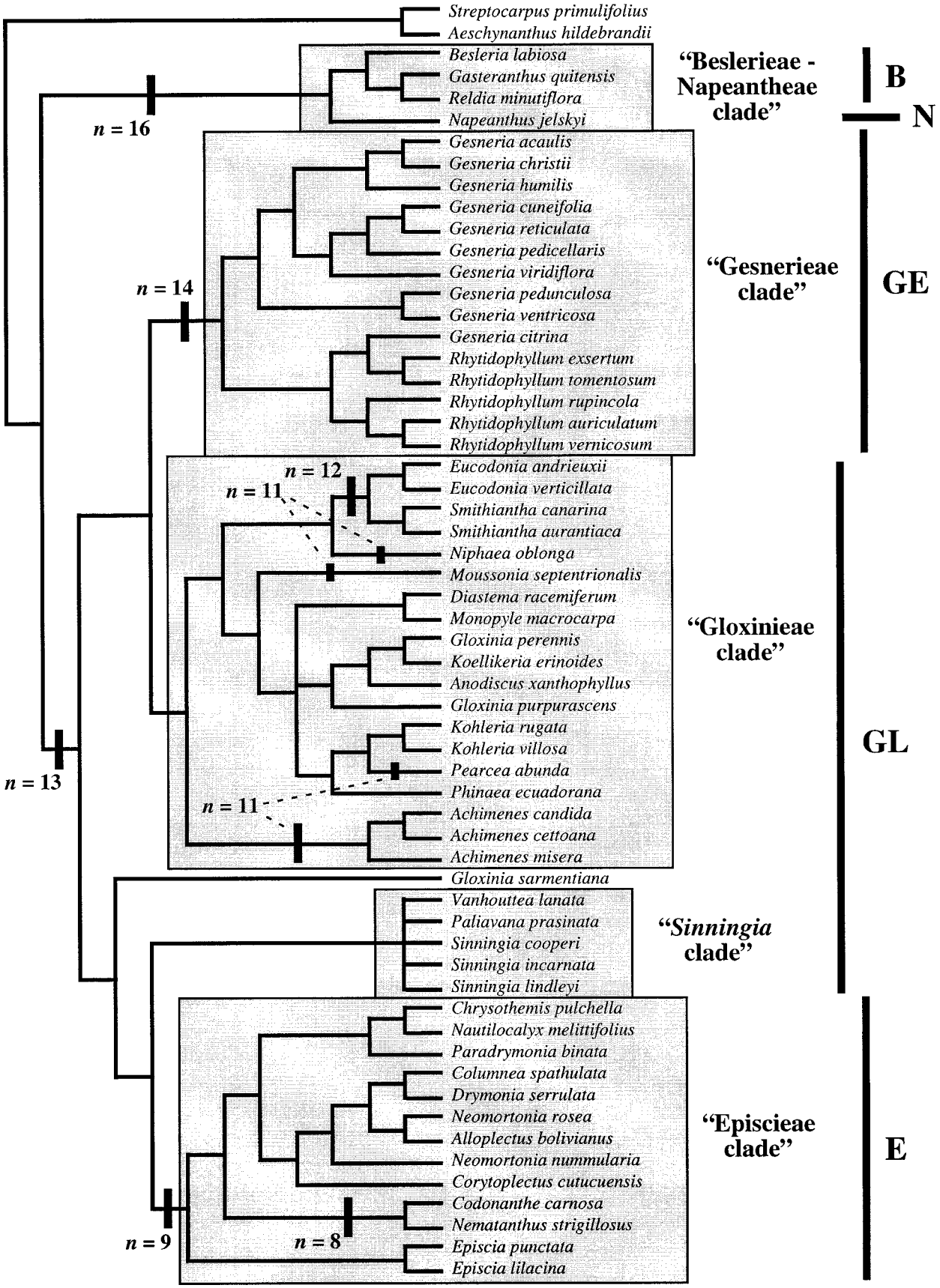


Fig. 5. Gesnerioideae *trnL-F*/*trnE-T* maximum likelihood tree (−ln = 7402.75877). Details of model parameters are listed in Table 1.


Streptocarpus primulifolius
Aeschynanthus hildebrandii
Besleria labiosa
Gasteranthus quitensis
Reldia minutiflora
Napeanthus jelskyi
"Beslerieae - Napeantheae clade"
B
N
n = 16
Gesneria acaulis
Gesneria christii
Gesneria humilis
Gesneria cuneifolia
Gesneria reticulata
Gesneria pedicellaris
Gesneria viridiflora
Gesneria pedunculosa
Gesneria ventricosa
Gesneria citrina
Rhytidophyllum exsertum
Rhytidophyllum tomentosum
Rhytidophyllum rupincola
Rhytidophyllum auriculatum
Rhytidophyllum vernicosum
"Gesnerieae clade"
GE
n = 14
n = 12
n = 11
Eucodonia andrieuxii
Eucodonia verticillata
Smithiantha canarina
Smithiantha aurantiaca
Niphaea oblonga
Moussonia septentrionalis
Diastema racemiferum
Monopyle macrocarpa
Gloxinia perennis
Koellikeria erinoides
Anodiscus xanthophyllus
Gloxinia purpurascens
Kohleria rugata
Kohleria villosa
Pearcea abunda
Phinaea ecuadorana
Achimenes candida
Achimenes cettoana
Achimenes misera
"Gloxinieae clade"
GL
n = 13
n = 11
Gloxinia sarmentiana
Vanhouttea lanata
Paliavana prasinata
Sinningia cooperi
Sinningia incarnata
Sinningia lindleyi
"Sinningia clade"
Chrysothemis pulchella
Nautilocalyx melittifolius
Paradrymonia binata
Columnea spathulata
Drymonia serrulata
Neomortonia rosea
Alloplectus bolivianus
Neomortonia nummularia
Corytoplectus cutucuensis
Codonanthe carnosa
Nematanthus strigillosus
Episcia punctata
Episcia lilacina
"Episcieae clade"
E
n = 9
n = 8

dunculosa (DC.) Fritsch + *G. ventricosa* Sw. (jk = 97%), and Group 3 comprises *G. citrina* + *R. rupincola* (C.Wright) C.V.Morton + *R. auriculatum* Hook. + *R. exsertum* Griseb. + *R. tomentosum* (L.) Mart. + *R. vernicosum* (jk = 74%; Fig. 3).

Group 1 species were all included in *Gesneria* section *Physcophyllon* L.E. Skog (Skog, 1976), distinguished by their nearly stemless habit, inflorescences shorter than the leaves, and usually red or reddish corollas. The species in Group 2 were included in two sections [*Pentarhaphia* (Lindl.) Fritsch, *G. ventricosa*; *Dittanthera* (G. Don) L.E. Skog, *G. pedunculosa*] of *Gesneria*. Both sections include species with similar habit (erect, shrubby, resinous plants) but are different in floral characteristics (Skog, 1976). Group 3 is more problematic from a morphological point of view, as it includes *G. citrina*, a species that had always been included among typical species of *Gesneria*. This species is a pendent plant in the wild, with small plane leaves and bright yellow tubular flowers, very different from the typical shrubby species of *Rhytidophyllum* with large bullate or areolate leaves and red, green, or rarely yellow campanulate corollas (Skog, 1976).

Relationships within the Gloxinieae—While the Gloxinieae sensu stricto is strongly supported as monophyletic with the exclusion of the *Sinningia* clade and *Gloxinia sarmentiana*, how the genera are related is not entirely clear (Fig. 3). Five moderately to strongly supported clades are evident with two orphan genera not strongly supported as associated with these clades. *Achimenes* is moderately supported as monophyletic (jk = 77%). The three species included (*A. candida* Lindl., *A. cettoana* H.E. Moore, and *A. misera* Lindl.) represent the three main clades found in more detailed studies of this genus (E. H. Roalson, unpublished data). *Achimenes* is weakly supported as being sister to the rest of the Gloxinieae sensu stricto clade (jk < 50%).

The genera *Eucodonia* Hanst. and *Smithiantha* Kuntze are strongly supported as monophyletic (for *Eucodonia*, jk = 100%; for *Smithiantha*, jk = 99%) and as sister genera (jk = 100%) and are weakly supported as sister to *Niphaea* (jk < 50%). Additionally, *Eucodonia* and *Smithiantha* are the only genera of Gesnerioideae to have an $n = 12$ chromosome complement. These genera both include rhizomatous rosette plants distributed in Central America.

Diastema and *Monopyle* form a moderately supported clade (jk = 74%). Morphological studies have not suggested that these two genera are closely related (Wiehler, 1983). Previous molecular phylogenetic studies using *ndhF* have placed *Diastema* and *Monopyle* in a clade with *Solenophora* Benth. (not sampled here; Smith et al., 1997b; Smith and Atkinson, 1998), although support for this clade was weak (Smith et al., 1997b: decay = 1; Smith and Atkinson, 1998: bootstrap = 14%).

Gloxinia (excluding *G. sarmentiana*), *Anodiscus*, and *Koellikeria* form a well-supported clade (Fig. 3; jk = 89%). The two species of *Gloxinia* sampled in this clade represent typical *Gloxinia* (*G. perennis*) and the segregate genus *Seemannia* Regel (*G. purpurascens*). *Gloxinia perennis*, *Anodiscus*, and *Koellikeria* share a raceme-like flowering stem (''inflorescence''), with flowers solitary in the axils of strongly reduced leaves, and the grouping of these three taxa is strongly supported (jk = 95%). Within this clade, *Gloxinia* is strongly supported as paraphyletic (jk = 95%). These data might suggest that *Seemannia* should be recognized as a distinct genus. Pollen stainability of intergeneric hybrids was a strong impetus for the combination of *Seemannia* with *Gloxinia*; crossing attempts between *Anodiscus* and other genera of the Gloxinieae have not been reported (for information on crossing studies see Wiehler [1976]). One cross has been successful between *Gloxinia perennis* and *Koellikeria erinoides* (Roberts, 1985), but no indication of pollen viability was offered. Additionally, while some pollen viability (deduced from partial fertility of the hybrids) was found in crosses between *Seemannia* and *Gloxinia*, pollen stainability was much less than that among other congeneric crosses: 19% was found in *Seemannia latifolia* Fritsch × *Gloxinia perennis* and 20% in *Seemannia latifolia* × *Gloxinia lindeniana* (Regel) Fritsch vs. 79% in *Gloxinia perennis* × *G. gymnostoma* Griseb. and 100% in *Nautilocalyx panamensis* (Seem.) Seem. × *N. villosus* (Kunth & Bouché) Sprague (Wiehler, 1976). While many interspecific crosses resulted in 0% pollen stainability, some did not: 5–8% was found in *Diastema vexans* H.E. Moore × *Kohleria spicata* (Kunth) Oerst., 0–11% in *Koellikeria erinoides* × *Kohleria spicata*, and 8% in *Seemannia latifolia* × *Kohleria spicata* (Wiehler, 1976), but these crosses with partial pollen stainability were not subsumed into one genus.

Previous phylogenetic studies have only sampled one species of *Gloxinia* (*G. sylvatica* = *Seemannia*) and had placed it as sister to *Niphaea* (Smith et al., 1997b; Smith and Atkinson, 1998). One of these studies also sampled *Anodiscus* and *Koellikeria* (Smith and Atkinson, 1998), but did not support the grouping of these three genera.

Kohleria Regel and *Pearcea* Regel have both undergone revision (Kvist and Skog, 1992, 1996). Currently, *Pearcea* is considered to include Wiehler's *Parakohleria* (Wiehler, 1983; Kvist and Skog, 1996). In the analyses presented here, *Pearcea* and *Kohleria* form a strongly supported clade (jk = 100%; Fig. 3). Additionally, the node grouping the two samples of *Kohleria* separate from the *Pearcea* sample is strongly supported (jk = 100%; Fig. 3). The close affinity of these genera is congruent with traditional taxonomy that often considered these taxa as congeneric (Kvist and Skog [1992, 1996] and references therein).

The chromosome complement of *Pearcea* (as *Parakohleria*) was originally considered to be $n = 13$ (undocumented count; Wiehler, 1978), but more recent studies have shown an $n = 11$ chromosome complement for at least one species (*P. abunda*; Kvist and Skog, 1996). *Pearcea* appears to be the only Gesnerioideae genus with a primary distribution in South America and an $n = 11$ chromosome complement. All other $n = 11$ taxa (*Achimenes*, *Moussonia* Regel, and *Niphaea*), are primarily distributed in Mexico and Central America (Kvist

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Fig. 6. Suprageneric classification and chromosome evolution mapped onto the maximum parsimony combined data strict consensus tree. Inferred chromosomal changes are marked onto branches with black bars (base chromosome number). The suprageneric classification of Burtt and Wiehler (1995) and informal clade names (in quotes) are marked to the right of the species names. Abbreviations of Burtt and Wiehler's (1995) classification are as follows: B = Beslerieae, E = Episcieae, GE = Gesnerieae, GL = Gloxinieae, and N = Napeantheae.

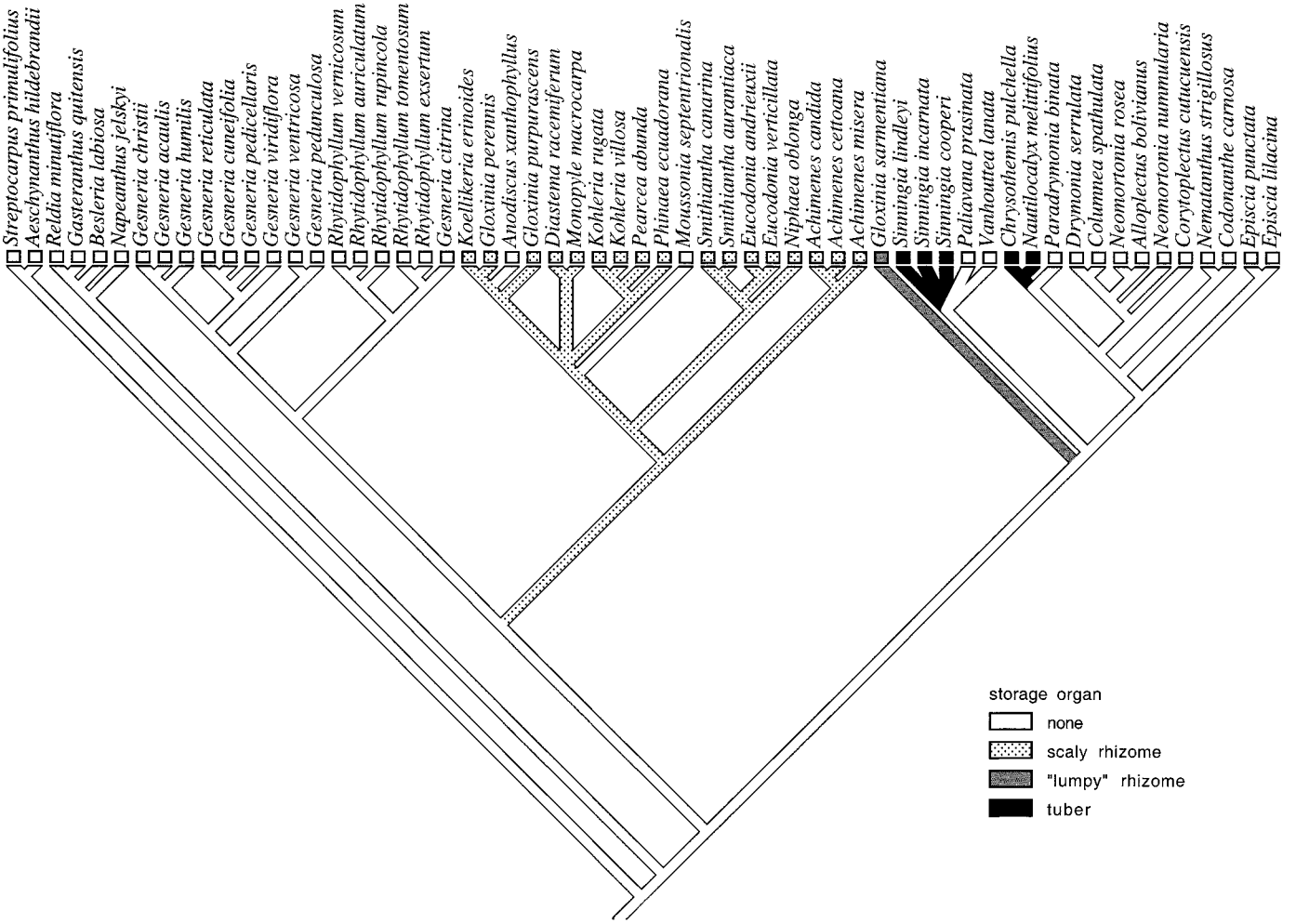

Fig. 7. Morphological characters mapped onto the maximum parsimony combined strict consensus tree. (A) Evolution of underground storage organs. (B) Evolution of ovary position.

and Skog, 1996). This close affinity of $n = 11$ and $n = 13$ genera separate from the Central American $n = 11$ genera suggests that the chromosome complement in the Gloxinieae may be more variable than it was previously considered.

In a previous study *Kohleria* was weakly grouped with *Achimenes* (bootstrap = 49%) while *Pearcea* was unresolved in relation to the *Kohleria*/*Achimenes* clade (Smith and Atkinson, 1998). Given the weak separation of these genera in the study by Smith and Atkinson (1998) and the strong statistical support of this clade in the current study, it appears that *Kohleria* and *Pearcea* are closely related.

The Sinningia clade and Gloxinia sarmentiana—While the grouping of *Sinningia*, *Paliavana*, and *Vanhouttea* is strongly supported (jk = 100%), relationships among these genera are not clear (Fig. 3). More detailed sampling of *Sinningia*, *Paliavana*, and *Vanhouttea* will be necessary to discern phylogenetic relationships among these genera, although morphological cladistic studies suggest *Paliavana* and *Vanhouttea* are nested within *Sinningia*, as currently circumscribed (Boggan, 1991).

Gloxinia sarmentiana is not closely related to other members of *Gloxinia*, although its specific affinity is unclear in our analyses. The ITS and combined analyses place *G. sarmentiana* as sister to a combined Episcieae/*Sinningia* clade (Figs. 1 and 3), while the cpDNA analysis places *G. sarmentiana* as sister to the *Sinningia* clade (Fig. 2). In either case, this species is not closely related to the other members of *Gloxinia* we sequenced. Interestingly, *G. sarmentiana* is somewhat separated geographically from most of the rest of *Gloxinia* (in southern Brazil vs. the Andes mountains for most of *Gloxinia*), and has a different storage organ type than typical *Gloxinia* species (it has a ''lumpy rhizome'' that is similar to tubers on a rhizome vs. a scaly rhizome for the rest of *Gloxinia*; Fritsch, 1900; J. K. Boggan, personal observation). Additional sampling of other *Gloxinia* species in southern Brazil may determine the extent of this separate lineage. Curiously, while *Gloxinia sarmentiana* is outside of the geographic range of typical *Gloxinia*, most of the species of *Sinningia* also occur in southern Brazil, a fact that may support phylogenetic relatedness among these taxa.

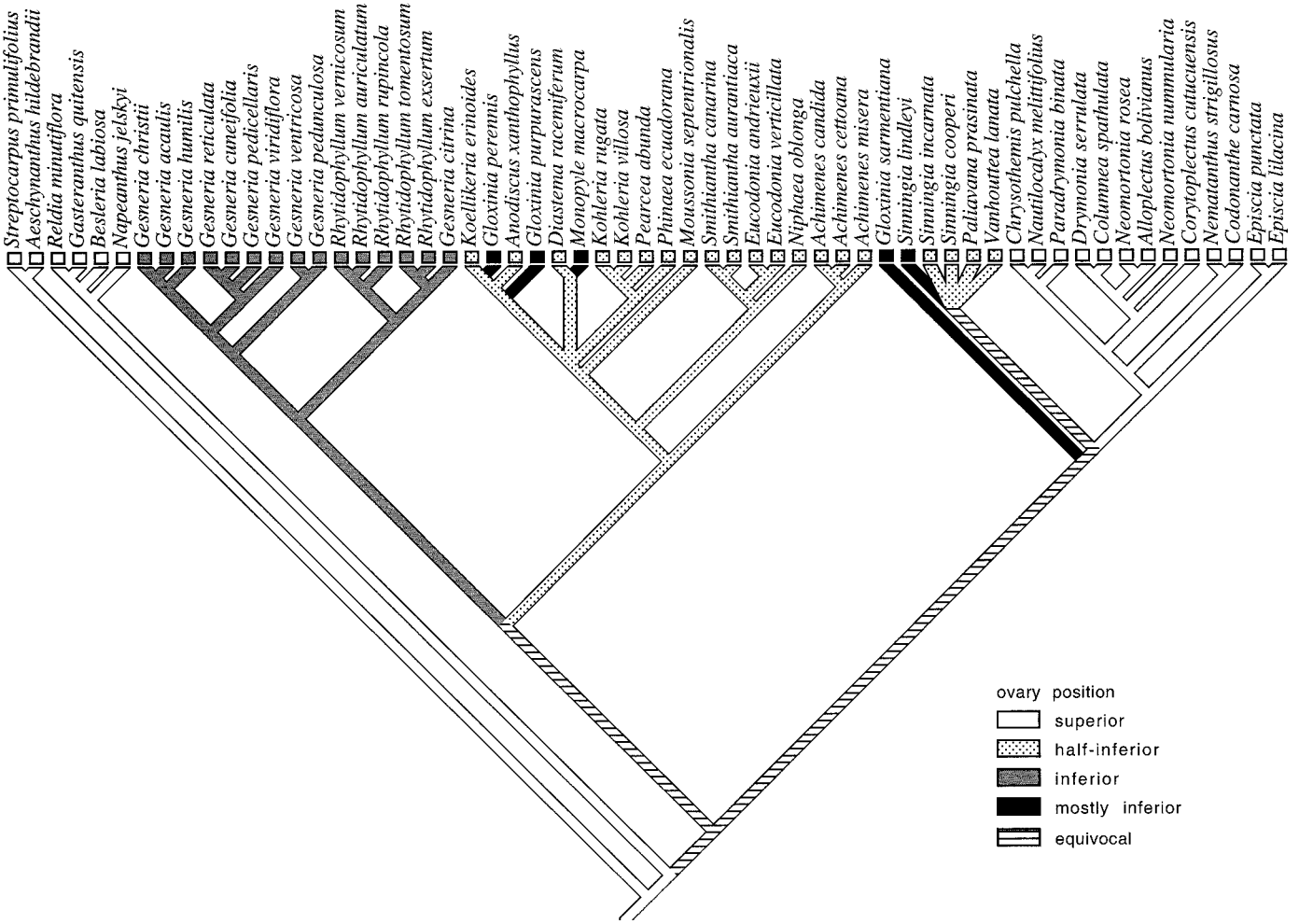

Fig. 7. Continued.

Chromosome evolution—Unlike the situation in Cyrtandroideae, chromosome number is fairly stable in the Gesnerioideae (Skog, 1984; Burtt and Wiehler, 1995). Base chromosome number is fairly congruent with the phylogenetic hypotheses presented here (Fig. 6). All $n = 14$ taxa (Gesnerieae) form a strongly supported clade (jk = 100%; Figs. 3 and 6), the $n = 12$ genera form a strongly supported clade (jk = 100%; Figs. 3 and 6), the $n = 9$ taxa (most of Episcieae) form a clade with $n = 8$ genera (*Codonanthe* and *Nematanthus*) that is nested within the $n = 9$ clade as a strongly supported clade (jk = 100%; Figs. 3 and 6). The $n = 13$ genera do not form a monophyletic group due to the separation of the *Sinningia* clade from the primary Gloxinieae clade and the close relationship of *Pearcea* ($n = 11$) with *Kohleria* ($n = 13$; Figs. 3 and 6). The other $n = 11$ genera (*Achimenes*, *Moussonia*, and *Niphaea*) do not form a clade, but are scattered among poorly supported branches holding the major groups of Gloxinieae together (Figs. 3 and 6). These data suggest that while chromosome complement across the Gesnerioideae is relatively stable, there are more changes in chromosome complement within the Gloxinieae than previously postulated.

Evolution of morphological characters—Although the majority of species in the Gesnerioideae have no specialized stem structures, there are two broad categories of underground stem storage structures present: tubers and scaly rhizomes (Boggan, 1991; Kvist and Skog, 1992). Scaly rhizomes appear to be synapomorphic for the Gloxinieae tribe (Fig. 7A). Tubers have arisen at least twice: once in the Episcieae genera *Chrysothemis* and *Nautilocalyx* and again in most species of the genus *Sinningia* (Fig. 7A). While traditionally considered a member of the Gloxinieae, *Gloxinia sarmentiana* does not have the typical scaly rhizome, but instead has what we refer to as a ''lumpy'' rhizome. This lumpy rhizome is similar in some regards to the tubers produced on underground rhizomes found in some species of *Sinningia* not yet sequenced (*S. curtiflora* (Malme) Chautems, *S. richii* Clayberg, and *S. tubiflora* (Hook.) Fritsch). With additional sampling of *Gloxinia* species from southern Brazil and species of *Sinningia* with tubers on rhizomes, a stronger relationship between these groups may be found, as suggested by the cpDNA analyses (Figs. 2 and 5).

While the ovary is typically superior in the Lamiales, ovary position in the Gesnerioideae appears to be quite variable; ova-

ries are superior, half-inferior, mostly inferior, or completely inferior (Fig. 7B). Given the combined data phylogenetic hypothesis, there have been either two or three changes from a superior to inferior ovary or one change of superior to inferior, with a subsequent reversion to superior ovaries in the Episcieae tribe (Fig. 7B). If the *Sinningia* clade and *Gloxinia sarmentiana* were sister to the Gloxinieae + Gesnerieae clade instead of sister to the Episcieae clade, one change from superior to inferior ovaries could be inferred. Since the node grouping the Episcieae clade, the *Sinningia* clade, and *Gloxinia sarmentiana* has low statistical support (jk $<$ 50%), the evolutionary sequence of ovary position change remains unclear.

Discordance between studies—As outlined above, there is discordance between this study and previously published phylogenetic hypotheses (Smith, 1996, 2000a, b; Smith and Carroll, 1997; Smith et al., 1997a, b; Smith and Atkinson, 1998). Some of this discordance can be attributed to the lower phylogenetic signal of the cpDNA *ndhF* gene sequences and morphology used previously in comparison to the noncoding nrDNA and cpDNA spacers we have sequenced.

One previous study, though, used ITS sequence data to explore relationships in the Episcieae (Smith, 2000b). The ITS sequences of the Episcieae included in this study provide statistical support to the monophyly of the Episcieae (jk = 81%) as well as major clades within the tribe, while those in Smith (2000b) do not (bootstrap = 6%). In the exploration of this issue, we note that all of the sequences in GenBank from the previous ITS study (Smith, 2000b) included a large percentage of ambiguously called bases (designated as an ''N''). As a means of more directly investigating these questions, comparisons were made between sequences of two species (*Gesneria christii* and *Niphaea oblonga* Lindl.) where the identical collection was sequenced for ITS in Smith (2000b) and this study (USBRG 94-507 and USBRG 78-354, respectively). There are significant differences in the sequences of the two species as cited in GenBank (*G. christii*, GBAN-AF206237; *N. oblonga*, GBAN-AF 206242) and the sequences produced in this study. The *Gesneria christii* raw sequence in GenBank differs from our sequence by 2.5% and ~23% of the sequence is designated by an ''N'' (or nucleic acid = unclear). Similarly, the raw sequence of *Niphaea oblonga* differs from our sequence by 3.8% and includes 22% Ns. In order to align the GenBank sequences to our data set, inferred gaps were often, but not always, necessary in our sequences where Ns are present in the GenBank sequences. In some cases, large amounts of nucleotide polymorphism have been found in ITS within individuals of Gesneriaceae species (Denduangboripant and Cronk, 2000). This does not appear to be the situation here, as our sequences were unambiguous and clean, with rare instances of single-base polymorphisms, but no indication of significantly different ITS copies being present.

Sequencing methodologies could possibly provide an explanation for some of the differences seen in the sequences. Our sequences were obtained using the ABI Prism BigDye Terminator Cycle Sequencing kit run on an Applied Biosystems Model 377 Automated sequencer with full overlap along both strands (PE Biosystems). This method of sequencing produces clean sequencing chromatograms, which, when both strands are sequenced, provide for confident base calling. The Smith (2000b) paper cites Smith et al. (1997b) for ITS sequencing methodologies, which were apparently the same as for the previous *ndhF* studies and utilized the Silver Sequence method (Promega). While this is an accepted method of sequencing, it does not provide for computer construction of consensus sequences from multiple strands (such as the Sequencher software package), which minimizes error in sequence reading and archiving. The explanation for the high frequency of missing data (Ns) is unclear. Smith (2000b) indicated that insertion/deletion (indel) events were coded as missing data, which could explain the Ns. However, GenBank notes that the sequences were not submitted as an aligned dataset, so there is no reason for indel events to be marked in the sequences. Additionally, the presence of the Ns aligned with our gaps do not explain the divergence between Smith's (2000b) and our sequences. Gaps do not contribute to uncorrected pairwise divergence estimates, so it is only sites where bases are called in both sequences that contribute to this measure.

Conclusions—This is the first study to provide robust statistical support of monophyly of (1) the Gesnerieae, (2) the Gloxinieae sensu stricto, (3) *Sinningia* and relatives, and (4) the Episcieae, as well as for the sister relationship of the Gesnerieae and Gloxinieae sensu stricto. Previous studies have suggested these clades were monophyletic but poorly supported, paraphyletic, or even polyphyletic (Smith, 1996, 2000a, b; Smith and Carroll, 1997; Smith et al., 1997a, b; Smith and Atkinson, 1998). With the exception of the distribution of the $n = 11$ taxa, the phylogenetic hypotheses presented here are also congruent with a minimal number of chromosomal changes in the subfamily. There are still problems with generic circumscription in some lineages (e.g., *Gloxinia*). While the Gesnerioideae tribal circumscriptions of Burtt and Wiehler (1995) largely agree with the phylogenies presented here, this study provides evidence that *Sinningia*, *Paliavana*, and *Vanhouttea* might be best dealt with as a tribe separate from the Gloxinieae. Additionally, *Gloxinia sarmentiana* may represent an additional major lineage of the Gesnerioideae. The molecular phylogenetic hypotheses do not completely agree with the traditional explanation for the evolution of some morphological characters (e.g., ovary position). The nodes placing the *Sinningia* clade and *Gloxinia sarmentiana* sister to the Episcieae rather than sister to the Gloxinieae and Gesnerieae needs to be explored to resolve this issue.

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