

PHYLOGENETIC ANALYSIS OF THE TRIBES GLOXINIEAE AND GESNERIEAE (GESNERIACEAE): DATA FROM NDHF SEQUENCES

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ABSTRACT. The neotropical Gloxinieae are one of the largest tribes in the subfamily Gesnerioideae (Gesneriaceae). The tribe contains 19 genera and approximately 230 species. The tribe Gesnerieae contains approximately 100 species with two to four genera depending on the treatment. Recent cladistic analyses of the tribes in the Gesneriaceae have indicated that *Sinningia*, *Paliavana*, and *Vanhouttea* are best removed from the Gloxinieae and placed in a separate tribe, Sinningieae. This study investigates the relationships of all but two of the remaining genera of the Gloxinieae, all but one of the Gesnerieae, and the relationship of the two tribes to each other. The Gloxinieae are found to be paraphyletic exclusive of the Sinningieae and the epiphytic genus *Capanea*. *Capanea* is found to be placed best within the Episcieae, a tribe that contains the majority of the epiphytic species in the neotropical Gesnerioideae. The Gloxinieae include a polyphyletic Gesnerieae with *Sanango* embedded in a polytomy of Gloxinieae and the remainder of the tribe monophyletic. The close sister group relationship of several genera is revealed and support is provided for the combination of *Parakohleria* into *Pearcea*. The two genera *Gesneria* and *Rytidophyllum* are in separate clades indicating that their separate generic status may be warranted.

INTRODUCTION

The Gesneriaceae comprise approximately 2500-3500 species in 120-135 genera, distributed primarily in the tropics with a few temperate species in Europe, China and Japan (Heywood 1978, A. Weber pers. comm.). The majority of species in the Gesneriaceae are herbaceous perennials, but can be annuals, shrubs, lianas, and trees. Many species (20%) are epiphytic, and the Gesneriaceae rank among the top ten plant families in terms of absolute numbers of epiphytic taxa (Madison 1977, Kress 1986). The family is divided into two or three subfamilies (Burt & Wiehler 1995, Smith *et al.* 1997a), the Gesnerioideae (including the subfamily Coronantheroideae as the tribe Coronanthereae) are primarily neotropical and the Cyrtandroideae primarily paleotropical. The Gesnerioideae are divided further into six tribes and 60 genera (Burt & Wiehler 1995, Smith *et al.* 1997a) and include the Gloxinieae and Gesnerieae.

The Gloxinieae are the largest tribe among the neotropical Gesneriaceae in terms of genera, comprising 19 genera under current classification systems (Burt & Wiehler 1995, Smith *et al.* 1997a) and approximately 230 species (Burt & Wiehler 1995). The tribe also represents a broad array of morphological diversity.

The Gloxinieae are united on the basis of morphological, anatomical and cytological data. The abaxial epidermal anticlinal cell walls of the leaves are strongly sinuate, stomata are

present in mounds, and the nodal anatomy is a vascular crescent (Wiehler 1983). In addition, the chromosome numbers of $n = 10$, 11, 12, and 13 (the latter being most prevalent) are not known among other members of the Gesnerioideae with the exception of $n = 13$ (Wiehler 1983).

Most species of Gloxinieae are herbaceous perennials of a terrestrial habit although the tribe has traditionally (Wiehler 1983, Burt & Wiehler 1995) contained a single epiphytic genus (*Capanea* Dcne. ex Planch.) and small trees (*Solenophora* Benth.). Additionally, some members of the tribe have adaptations to a seasonally dry habitat, such as tubers (*Lembocarpus* Leeuw.), and scaly rhizomes are widespread among members of the Gloxinieae (13 out of the 19 genera) (Wiehler 1983). It is the goal of this study to examine the phylogenetic relationships among the genera of this tribe and to determine if the monophyly of the tribe as proposed by Wiehler (1983) and Burt & Wiehler (1995), but excluding Sinningieae, is upheld. In addition, by providing a phylogenetic framework, it will be possible to examine the changes in chromosome numbers through this tribe. This study includes all genera of the Gloxinieae with the exception of *Lembocarpus* and *Goyazia* Taub.

The Gloxinieae as emended by Wiehler (1983) comprises five tribes of Fritsch (1893-94) (Bellonieae, Gloxinieae, Kohlerieae, Sinningieae, and Solenophoreae) included on the basis of hybridization experiments (Wiehler 1968, 1976a, 1976b). Hybrids with *Sinningia* were not formed in the experiments, although

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Wiehler (1983) included Sinningieae within the Gloxinieae on the basis of "the same general characteristics" and the fact that tubers, used to separate the Sinningieae from the Gloxinieae, were not present in all species of *Sinningia*. Recent cladistic analyses of morphology (Smith 1996) and comparative *ndhF* sequences (Smith *et al.* 1997a) indicate that the Sinningieae are best removed from the Gloxinieae and are thus in agreement with the hybridization studies.

The Gesnerieae are one of the smaller tribes within the Gesnerioideae comprising two (Burt & Wiehler 1995) or three (four if *Sanango* were included) (Skog 1976) genera. These plants are distinguished by alternate leaves in most species, specialized petiole vasculature, an annular nectary, and a distribution almost exclusively in the Caribbean (Skog 1976, Wiehler 1983). The one exception to the geographic distribution is the monospecific *Sanango* G. S. Bunting and J. A. Drake (Wiehler 1994). Skog (1976) in his treatment of the Gesnerieae, divided the tribe into three genera, *Gesneria* L., *Rytidophyllum* Mart., and *Pheidonocarpa* L. E. Skog, distinguishing the genera on the basis of stamen attachment to the corolla and several fruit characters such as presence of a beak on the capsule of *Pheidonocarpa* and costae present on the capsules of *Gesneria* (Skog 1976). Wiehler (1983) in his revision of the Gesnerioideae, combined these three genera into the single genus *Gesneria* on the basis that many of the characters used to separate the genera were no longer valid generic level characters in his new system (Wiehler 1983). *Sanango* was later added to this tribe after re-examination of several features of this Ecuadorean genus (Dickison 1994, Jensen 1994, Norman 1994, Wiehler 1994, Burt & Wiehler 1995, Smith *et al.* 1997b). This present analysis includes representatives of all lineages within the Gesnerieae (Skog 1976) with the exception of *Pheidonocarpa*.

The source of molecular data for this analysis is the chloroplast DNA encoded gene *ndhF*, which in tobacco encodes a protein of 740 amino acids presumed to be a subunit of an NADH dehydrogenase (Sugiura 1992). The use of *ndhF* sequences for systematic studies has provided a far greater number of characters to resolve relationships than studies using *rbcL* (Olmstead & Sweere 1994, Clark *et al.* 1995, Olmstead & Reeves 1995, Scotland *et al.* 1995, Smith *et al.* 1997a, 1997b). The larger number of variable characters makes *ndhF* sequences ideal for taxonomic groups that have not been resolved well using *rbcL* data, such as members of the Aster-

idae and Lamiales s.l. (Olmstead *et al.* 1992, 1993, Chase *et al.* 1993).

MATERIALS AND METHODS

The gene sequences used in this analysis were generated by thermal cycle sequencing (Innis *et al.* 1988) of previously amplified *ndhF* regions. The *ndhF* gene was amplified in two overlapping sections (positions 1–1,350, and 972–2,044) from genomic DNA isolated from fresh, frozen, or silica gel dried material (Smith *et al.* 1992). Amplification and sequencing procedures followed that of Smith *et al.* (1997a) used for other members of the Gesneriaceae.

The focus of this analysis was on the relationships within the tribes Gloxinieae and Gesnerieae and the relationship between these two tribes, therefore every attempt was made to gather all genera within the two tribes. The only members of the Gloxinieae excluded from this analysis are the rare, Surinam endemic *Lembo-carpus*, and the Brazilian endemic *Goyazia*. The only Gesnerieae excluded was *Pheidonocarpa*. The species used in the analysis, voucher information, and Genbank accession numbers are included in TABLE 1.

Outgroups were selected to root the tree based on recent analyses of tribal relationships within the Gesneriaceae (Smith 1996, Smith *et al.* 1997a). The most appropriate outgroups for the tribes Gloxinieae and Gesnerieae were genera representing the other tribes of the Gesnerioideae. Previous cladistic analyses of Gesneriaceae (Smith 1996, Smith *et al.* 1997a, 1997b) and within Gesnerioideae (Smith & Carroll in press) have indicated that the Coronanthereae are sister to the remainder of the Gesnerioideae and were therefore used as the designated outgroup in this analysis.

Sequences were aligned by hand to the sequence for *Nicotiana* L. and all indels with respect to *Nicotiana* were excluded from the phylogenetic analysis.

PHYLOGENETIC ANALYSIS. Phylogenetic divergence was reconstructed using PAUP version 3.1.1 (Swofford 1993) to implement parsimony (Farris 1970, Farris *et al.* 1970, Swofford & Maddison 1987). In this analysis, trees were generated using the general heuristic option, saving minimal trees only, with the collapse zero-length branches, and ignore uninformative characters options in effect. Because of the large number of taxa in this analysis, the branch and bound and exhaustive search options would have consumed an excessive amount of time. Therefore, the trees presented here are best approximations and not exact solutions. The manner in

TABLE 1. Species sequenced in this study with Genbank submission numbers and voucher specimens. SI—Smithsonian Institution living collection, LG—Longwood Gardens. Letters in parentheses indicate herbarium acronyms where vouchers are deposited.

Species	Voucher	Genbank number
<i>Achimenes skinneri</i> Lindl.	SI 94-606	U62177
<i>Alloplectus panamensis</i> C. V. Morton	SI 93-060	AF013685
<i>Alloplectus</i> sp.	Smith 3418 (SRP)	AF013686
<i>Alsobia dianthiflora</i> (H. E. Moore & R. G. Wilson) Wiehler	SI 94-258	AF013687
<i>A. punctata</i> (Lindl.) Wiehler	Smith 3600 (SRP)	AF013688
<i>Alsobia</i> sp.	SI 94-005	AF013689
<i>Alsobia</i> sp.	Smith 3599 (SRP)	AF013690
<i>Anodiscus xanthophyllus</i> (Poepp.) Mansf.	Dunn s.n. (SRP)	AF040143
<i>Bellonia</i> sp.	Evans s.n. (SRP)	AF040144
<i>Besleria affinis</i> Morton	LG870575	U62162
<i>Capanea grandiflora</i> (Kunth) Decne. ex Planch.	Smith 1199 (WIS)	AF040145
<i>Chrysothemis friedrichsthaliana</i> (Hanst.) H. E. Moore	SI 94-335	AF013691
<i>Cobonanthus calochlamys</i> (J. D. Sm.) Wiehler	SI 94-421	AF013692
<i>Codonanthe elegans</i> Wiehler	SI 82-45	U62178
<i>Codonanthis peruviana</i> Wiehler	Turley s.n. (SRP)	AF013693
<i>Columnnea ambigua</i> (Urban) Morley	Smith 3701 (SRP)	AF013694
<i>C. mira</i> Morley	Smith 2450 (WIS)	AF013695
<i>C. oblongifolia</i> Rusby	Smith 1721 (WIS)	AF013696
<i>C. sanguinea</i> (Pers.) Hanst.	Smith 3369 (SRP)	AF013697
<i>Columnnea schiedeana</i> Schlecht.	Smith 288 (WIS)	U62164
<i>Corytoplectus speciosus</i> (Poepp.) Wiehler	SI 94-268	AF013698
<i>Diastema racemiferum</i> Benth.	SI 85-98	U62156
<i>Drymonia urceolata</i> Wiehler	Smith 3416 (SRP)	AF013699
<i>Episcia fimbriata</i> Fritsch	SI 95-005	AF013700
<i>E. sphalera</i> Leeuw.	SI 94-043	AF013701
<i>Eucodonia andrieuxii</i> (DC.) Wiehler	Dunn s.n. (SRP)	AF040146
<i>Gasteranthus corallinus</i> (Fritsch) Wiehler	SI 94-243	U62163
<i>Gesneria pedicellaris</i> Alain	SI 94-567	U62192
<i>Gesneria christii</i> Urban	SI 94-507	U62191
<i>Gloxinia sylvatica</i> (H.B.K.) Kunth	Dunn 9012051 (SRP)	U62157
<i>Heppiella ulmifolia</i> (Kunth) Hanst.	Smith 3427 (SRP)	AF040147
<i>Koellikeria erinoides</i> (DC.) Mansf.	Dunn s.n. (SRP)	AF013709
<i>Kohleria spicata</i> (Kunth) Oerst.	SI 94-552	U62181
<i>Monopyle macrocarpa</i> Benth.	No voucher	U62197
<i>Moussonia strigosa</i> (C. V. Morton)	Dunn s.n. (SRP)	AF040148
<i>Napeanthus costaricensis</i> Wiehler	No voucher	U62198
<i>Napeanthus macrostoma</i> Leeuwenberg	Feuillet (US)	U62161
<i>Nautilocalyx adenosiphon</i> (Leeuw.) Wiehler	SI 93-017	AF013702
<i>Nematanthus hirsutus</i> (Mart.) Wiehler	Marie Selby B. G.	L36404
<i>Neomortonia nummularia</i> (Hanst.) Wiehler	SI 94-469	AF013703
<i>N. rosea</i> Wiehler	SI 94-230	AF013704
<i>Niphaea oblonga</i> Lindl.	SI 78-354	U62160
<i>Paradrymonia aurea</i> Wiehler	SI 94-474	AF013705
<i>P. densa</i> (C. H. Wright) Wiehler	Stewart s.n. (SRP)	AF013706
<i>P. fuquaiana</i> Wiehler	SI 93-027	AF013707
<i>Pearcea</i> sp.	Smith 3425 (SRP)	AF040149
<i>P. hypocyrtiflora</i> Regel	SI 83-082	AF040150
<i>Phinaea albiflora</i> Rusby	SI 94-503	AF040151
<i>Rufodorsia major</i> Wiehler	SI 94-575	AF013708
<i>Rytidophyllum tomentosum</i> (L.) Mart.	SI 77-235	U62200
<i>Rytidophyllum auriculatum</i> Hook.	SI 94-524	U62199
<i>Sanango racemosum</i> (R. & P.) Barringer	Wiehler (GRF)	U62144
<i>Smithiantha cinnabarina</i> (Linden) Kuntze	SI 94-484	AF040152
<i>Solenophora obliqua</i> D. L. Denham & D. N. Gibson	Breedlove 71542 (CAS)	U62202
Outgroups		
<i>Asteranthera ovata</i> (Cav.) Hanst.	Stewart 12234 (SRP)	U62204
<i>Fieldia australis</i> Cunn.	Stewart s.n. (SRP)	U62196
<i>Mitraria coccinea</i> Cav.	Stewart s.n. (SRP)	U62193
<i>Negria rhabdothamnoides</i> F. Muell.	Nordenstam 8608 (S)	U62195
<i>Paliavana prasinata</i> (Ker-Gawl.) Fritsch	SI 78-368	U62174

TABLE 1. Continued.

Species	Voucher	Genbank number
<i>Sarmienta repens</i> R. & P.	Stewart s.n. (SRP)	U62194
<i>Sinningia (Lietzia) brasiliensis</i> (Regel & Schmidt) Wiehler	Dunn 9104014 (SRP)	U62175
<i>Sinningia cooperi</i> (Paxt.) Wiehler	SI 94-340	U62201
<i>Sinningia richii</i> Clayb.	SI 94-554	U62186
<i>Vanhouttea lanata</i> Fritsch	SI 94-516	U62203

which the program reconstructs phylogenetic sequences is sensitive to the order of taxa presentation in the data matrix, frequently finding islands of equally-parsimonious trees depending on the order of taxon input (Maddison 1991). Therefore, it is important to repeat the analysis several times. To do this, the search strategy of Olmstead & Palmer (1994) was implemented searching for 1,000 trees each with a random order of taxa in the data matrix in five subsequent analyses with the nearest neighbor interchange (NNI) search option in effect and mulpars "off." Each of the results from the five NNI searches was used as the starting tree(s) for a search with tree bisection reconnection (TBR) and mulpars "on." The same strategy was used with the constraints option of PAUP to test for monophyletic groups.

Branch support analysis (decay) was performed to examine trees that were longer than the most-parsimonious trees (Bremer 1988, 1994, Donoghue *et al.* 1992). This type of analysis provides an indication of the robustness of the data by determining which clades persist in a consensus tree as parsimony is relaxed. This analysis was performed by saving all trees six steps longer than the most-parsimonious trees and then examining subsets of trees one to five steps longer with the filter option of PAUP. Decay values greater than five steps were determined with the constraints option of PAUP, searching for the shortest tree that did not contain that clade.

Bootstrap analysis (Felsenstein 1985) was performed using 100 replicates with the TBR search option and mulpars "off." An estimate of how well the data fit the tree was determined with the g_i statistic (Hillis & Huelsenbeck 1992) using 10,000 random trees.

The monophyly of various tribal relationships not obtained in the most-parsimonious trees was examined by using the constraints option of PAUP. The constraints option was used to examine the monophyly of Gesnerieae and Gloxinieae as separate tribes and to determine the number of additional steps necessary to place *Capanea* in the Gloxinieae.

RESULTS

Two thousand two hundred twenty-three base pairs of *ndhF* were sequenced for each species in this analysis. Of these base pairs, 525 were variable and phylogenetically informative and another 175 were autapomorphic among the species used in this analysis (TABLE 1). An insertion at position 1,440 of 11 base pairs was found in all species of Gesnerioideae examined. The sequence of this insertion was not used in the cladistic analysis since it is constant among all species examined.

The cladistic analysis, using members of the Coronanthereae as the outgroup, resulted in three most-parsimonious trees of 3,514 steps each, consistency index (CI) = 0.27, retention index (RI) = 0.36. The strict consensus of these trees is presented in FIGURE 1 and one of the three trees, selected at random, is presented in FIGURE 2. Gesnerieae and Gloxinieae (excluding *Capanea*) together represent an inclusive monophyletic group. Most Gloxinieae are in a single clade, however *Bellonia* (Plum.) L. and *Smitthiantha* Kuntze are sister to each other and jointly are sister to the Gesnerieae, and *Capanea* is within the Episcieae (FIGURE 1). Nineteen additional steps beyond the most-parsimonious trees are necessary to place *Capanea* within the Gloxinieae. The two species of *Pearcea* Regel, including one unidentified species that previously would have been placed in *Parakohleria* Wiehler, form a monophyletic group (FIGURE 1), providing evidence for the combination of *Parakohleria* into *Pearcea* (Kvist & Skog 1996). *Gesneria* and *Rytidophyllum* each form separate clades (FIGURE 1). The origin of chromosome numbers and scaly rhizomes are mapped onto the strict consensus tree (FIGURE 1).

An analysis that constrained the monophyly of both Gesnerieae and Gloxinieae, excluding Gesnerieae, resulted in four trees that were three steps longer than the most-parsimonious trees (FIGURE 3). The effect of constraining both Gesnerieae and Gloxinieae as monophyletic had no effect on the remaining portions of the tree, therefore, only the Gesnerieae and Gloxinieae are

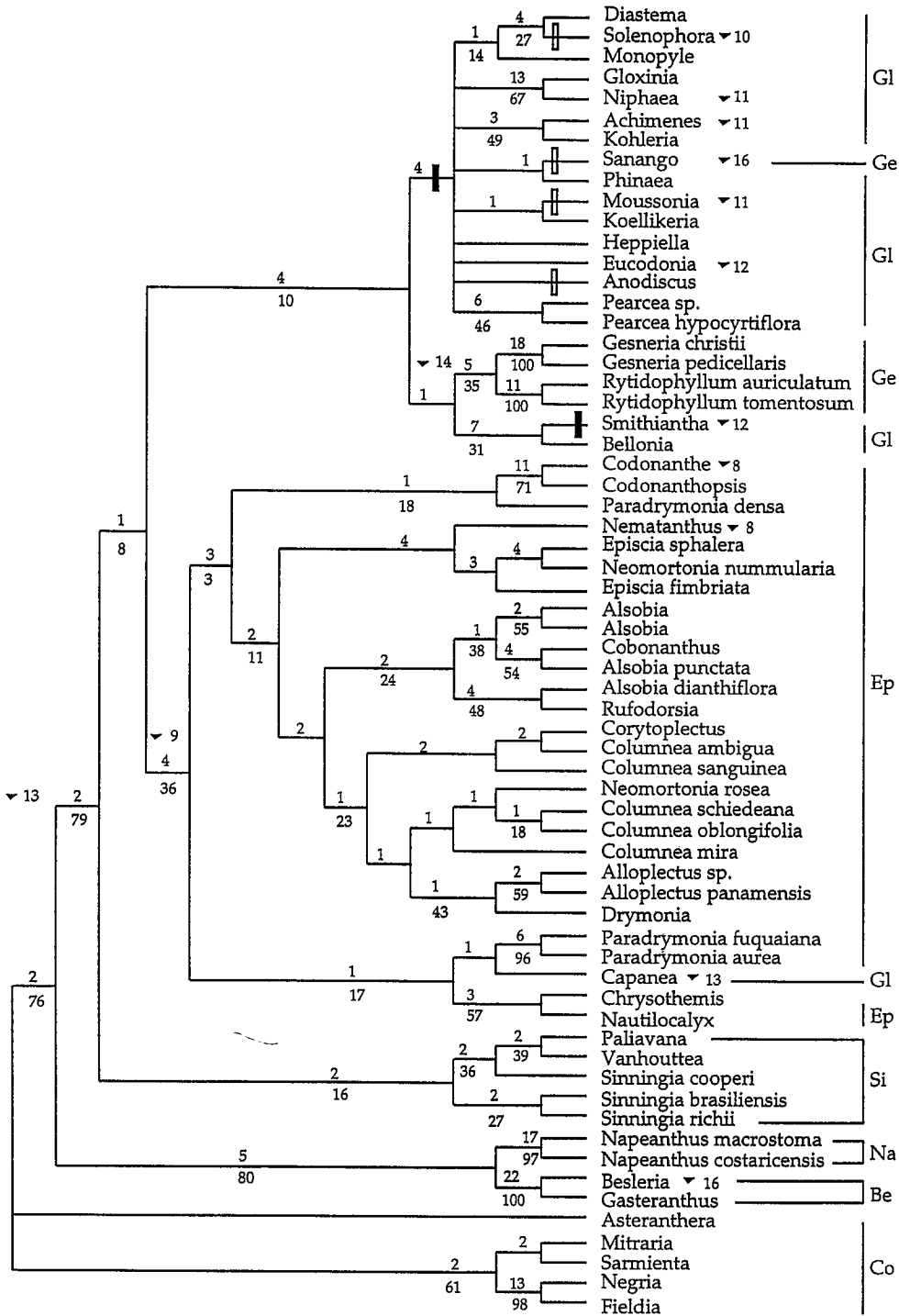


FIGURE 1. Strict consensus of three most-parsimonious trees of 3,514 steps each (CI = 0.27, RI = 0.36) based on *ndhF* sequences and rooted with the tribe Coronanthereae. Numbers below the clades are bootstrap values, numbers above are decay values. Clades without bootstrap values are those not found in the strict consensus of the bootstrap analysis. The Gloxinieae are monophyletic only if they contain the Gesnerieae and exclude *Capanea*. Tribal abbreviations are as follows: Be—Beslerieae, Co—Coronanthereae, Ep—Episcieae,

presented in FIGURE 3. The gain and loss of scaly rhizomes are mapped onto this consensus tree (FIGURE 3). An analysis that constrained only the monophyly of the Gesnerieae resulted in three trees one step longer than the most-parsimonious tree. In the strict consensus of these three trees, the Gloxinieae are still paraphyletic with *Smithiantha/Bellonia* as sister to the Gesnerieae and the Gesnerieae as sister to the Gesnerioideae except Sinningieae, Napeantheae, and Beslerieae (tree not shown).

There is significantly more structure than random data within this analysis as reflected by the g_i statistic (-0.237). If this value is extrapolated from the charts generated by Hillis and Huel- senbeck (1992), it is significant at $P < 0.01$.

DISCUSSION

GLOXINIEAE. The Gloxinieae, as delimited by Wiehler (1983) and Burt & Wiehler (1995), represent the combination of five tribes from previous classification systems (Fritsch 1893-4, Ivanina 1965). The combination of these five tribes into a single more-inclusive tribe was based largely on hybridization studies, in which numerous "inter-tribal" hybrids were generated (Wiehler 1983). Although hybrids between these tribes and the Sinningieae were not generated, Wiehler (1983) combined the Sinningieae into the Gloxinieae on the basis of "the same general characteristics." Previous cladistic analyses of the Gesneriaceae (Smith 1996, Smith *et al.* 1997a) and this analysis indicate the Sinningieae are best considered a separate tribe from the Gloxinieae. In addition to the hybridization studies, Wiehler (1983) cites the presence of 1) strongly sinuate anticlinal walls of the cells of the abaxial epidermis of the leaf, 2) presence of stomatal mounds, and 3) the same vascular crescent as characteristics uniting the genera of his Gloxinieae. Lastly the presence of chromosome numbers of $n = 10, 11, 12,$ and 13 was an additional factor in uniting these genera. An additional character, not discussed by Wiehler (1983) specifically in relation to the monophyly of the Gloxinieae, is the presence of scaly rhizomes in 13 of the 19 genera (Wiehler 1983). Although scaly rhizomes are known in some members of the subfamily Cyrtandroideae (*Briggsia* Craib, *Titanotrichum* Solereder) they are not known from other members of the Gesnerioideae.

The single epiphytic genus traditionally placed within the Gloxinieae, *Capanea*, belongs in the more broadly epiphytic tribe Episcieae based on *ndhF* sequences presented here (FIGURE 1). The presence of *Capanea* in the Episcieae will make it the only member of that tribe with a chromosome number of $n = 13$ whereas all other members are $n = 8$ or 9 . However, the placement of *Capanea* within the Episcieae is strongly supported by this analysis indicating that, while chromosome counts may provide a generalized pattern of evolution within the Gesneriaceae, they are also subject to convergence and parallelism as are other characters (Smith 1996, Smith *et al.* 1997a; Smith & Carroll in press).

The remainder of the Gloxinieae (with the possible exception of *Lembocarpus* and *Goyazia*, which have not been included in the analysis) are paraphyletic. This tribe is monophyletic only if it includes another tribe, the Gesnerieae (FIGURE 1). The inclusion of the Gesnerieae within the Gloxinieae may be an artifact of the analysis, because trees only three steps longer find separate monophyletic Gesnerieae and Gloxinieae (FIGURE 3). An analysis of all tribes of the Gesneriaceae, while sampling less within each tribe, identified these two tribes as separate, monophyletic groups (Smith *et al.* 1997a). An analysis of morphology indicated that the Gesnerieae were monophyletic but were sister to one clade of the Gloxinieae on the basis of a semi-inferior ovary common to all members of Gesnerieae and a portion of Gloxinieae (Smith 1996). Unfortunately, the two genera that are sister to the Gesnerieae in this present study, *Bellonia* and *Smithiantha*, were not included in the morphological analysis, however the two genera that created a paraphyletic Gloxinieae in the morphological analysis were *Monophyle* and *Solenophora*, both separate due to the lack of scaly rhizomes (a character state also shared with *Bellonia* but not *Smithiantha*). Both of these genera are in the main Gloxinieae clade in this analysis (FIGURE 1). It is possible that the Gloxinieae should be divided as it has been in previous classifications (Fritsch 1893-4, Ivanina 1965), although neither of these classification systems approximates the monophyletic groups identified here (FIGURE 1). A better division of the tribe would be the majority of genera traditionally placed in the Gloxinieae retained (FIG-

←
Ge—Gesnerieae, Gl—Gloxinieae, Na—Napeantheae, Si—Sinningieae. The origin of scaly rhizomes are marked with dark bars, the loss with white bars. Chromosome counts are marked with carats followed by the base number. Note that inferred chromosome numbers are indicated on some internal nodes.

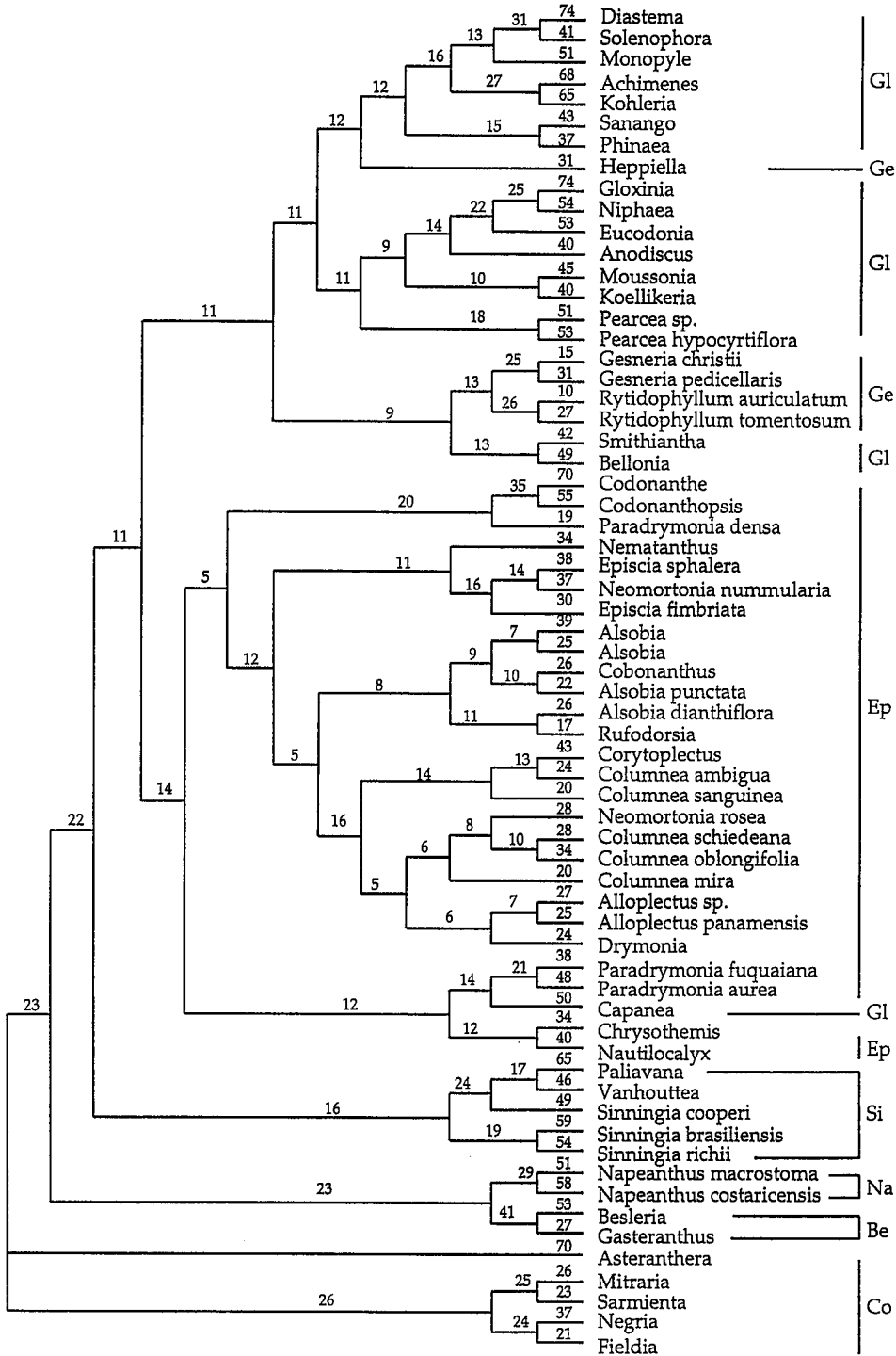


FIGURE 2. One of three most-parsimonious trees of 3,514 steps each (CI = 0.27, RI = 0.36) based on *ndhF* sequences and rooted with the tribe Coronanthereae. Numbers above the clades are branch lengths. Tribal abbreviations follow those of Figure 1.

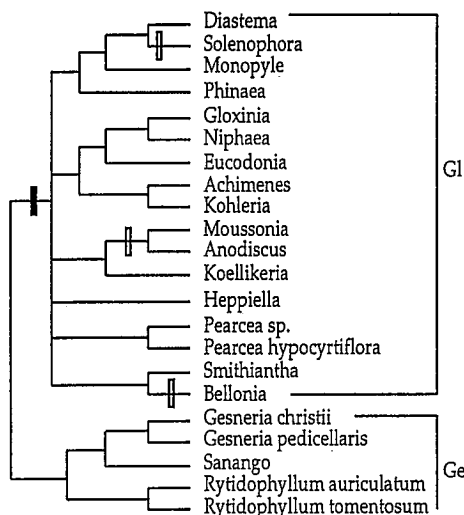


FIGURE 3. Strict consensus of four trees of 3,517 steps each where the tribes Gloxinieae and Gesnerieae were constrained to be separate and monophyletic showing only the Gloxinieae and Gesnerieae portions. Tribal abbreviations follow those of Figure 1. The origin of scaly rhizomes are indicated with dark bars and the loss with white bars.

URE 1) with *Bellonia* and *Smithiantha* removed to a separate tribe, Bellonieae (FIGURE 1).

The three additional steps longer than the most-parsimonious trees in the *ndhF* data necessary to make both Gloxinieae and Gesnerieae separate monophyletic tribes are offset by the decreased number of changes in the evolution of scaly rhizomes within the Gloxinieae. In the most-parsimonious trees, two gains and three losses of scaly rhizomes are necessary (FIGURE 1). However, in the tree where the monophyly of the two tribes is constrained, only a single gain and four losses are required since *Smithiantha* is now in a monophyletic Gloxinieae and *Moussonia* Regel and *Anodiscus* Benth. are sister genera (FIGURE 3). Therefore, this tree (FIGURE 3) may be a better approximation of the relationships of the tribes Gloxinieae and Gesnerieae as well as of the genera within them.

GESNERIEAE. The monophyly of Gesnerieae has never been disputed (Fritsch 1893–1894, Ivanina 1965, Skog 1976, Wiehler 1983) although the generic delimitations within the tribe have varied (Skog 1976, Wiehler 1983). The Gesnerieae have been considered to be distinct and monophyletic on the basis of chromosome number ($n = 14$), specialized petiole vasculature, alternate leaf arrangement in almost all species, and an annular nectary (Skog 1976, Wiehler 1983). In addition, most species are Carib-

bean in their distribution, the only exception being the monospecific *Sanango*, which has been demonstrated to be a member of the Gesnerieae through several lines of evidence (Dickison 1994, Jensen 1994, Norman 1994, Wiehler 1994, Smith *et al.* 1997b). However, *Sanango* is not within the Gesnerieae in this analysis and is instead in a polytomy within the Gloxinieae (FIGURE 1). The placement of *Sanango* within the Gloxinieae is likely to be spurious since only one additional step beyond the most-parsimonious trees is necessary to constrain *Sanango* to the Gesnerieae. The morphological, anatomical and chemical characters that provide support for its position in the Gesnerieae (Dickison 1994, Jensen 1994, Norman 1994, Wiehler 1994) offset the one *ndhF* character. Although Wiehler (1983) combined *Gesneria* and *Rytidophyllum* on the basis of not having sufficient character differences to be maintained as genera, the molecular data provide some support for the separate generic status of these two taxa (FIGURE 1) although sampling within each of these genera is minimal at this point.

ORIGIN OF CHARACTERS. The presence of scaly rhizomes within the Gloxinieae is unique among the Gesnerioideae, but based on the cladogram from this analysis (FIGURE 1) it is not a synapomorphic character uniting them. Genera that lack the scaly rhizomes (*Solenophora*, *Moussonia*, *Anodiscus*, and *Bellonia*) are dispersed among the scaly rhizomatous genera and do not form a monophyletic group among themselves. However, when Gloxinieae are constrained to be monophyletic, excluding Gesnerieae, scaly rhizomes have a single origin with only three losses in *Moussonia/Anodiscus*, *Bellonia*, and *Solenophora* (FIGURE 3).

The only genus of the Gloxinieae to possess tubers, *Lembocarpus*, was not available for this analysis. The presence of *Lembocarpus* within the Gloxinieae may well be questioned since tubers are known from tribes Sinningieae and Episcieae, but not other members of the Gloxinieae. The placement of *Lembocarpus* in Gloxinieae was based on putative hybrids generated between *Lembocarpus* and *Sinningia* Nees (Wiehler 1983); a report that later turned out to be premature (Boggan 1991). *Lembocarpus* also is the only genus in Gloxinieae to have a superior ovary, a character state more common within Episcieae. Further analyses will necessarily include *Lembocarpus* to see if it has a more appropriate tribal placement elsewhere, or perhaps an isolated position within the Gesnerioideae as indicated by its unique combination of seed characters (Beaufort-Murphy 1983).

Although members of the Gloxinieae (exclu-

sive of *Capanea*, which is now more appropriately viewed as *Episcieae*) are the only members of the Gesnerioideae to have chromosome counts of $n = 10, 11, 12$ these different counts do not serve as synapomorphies within the tribe (FIGURE 1). On the contrary, the count of $n = 13$ (the most prevalent count within *Gloxinieae*) probably is symplesiomorphic for the subfamily Gesnerioideae (FIGURE 1). The count of $n = 10$ in *Solenophora* is autapomorphic within the Gesnerioideae, although it is known from Cyrtandroideae (Skog 1984). Three genera are known to have counts of $n = 11$, *Niphaea* Lindl., *Achimenes* Pers., and *Moussonia*. This character is not synapomorphic for these three genera (FIGURE 1). *Smithiantha* and *Eucondonia* Hanst. are the only genera to share a count of $n = 12$ and likewise are not sisters in this analysis (FIGURE 1). Two genera of the traditional (Burt & Wiehler 1995, Wiehler 1983) *Gloxinieae* have not been counted; these are *Goyazia* and *Lembocarpus*. Unfortunately, neither of these two genera were available for this analysis, and have not been examined cladistically (see above for discussion of *Lembocarpus*). The apparent multiple origins of some chromosome counts in this (FIGURE 1) and other cladistic analyses of the Gesneriaceae (Smith 1996, Smith *et al.* 1997a, Smith & Carroll in press) indicate that chromosome numbers may provide a general guideline for monophyly, but they also are subject to parallelism and should not be relied upon too heavily for classification purposes.

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