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Tribal Relationships in the Gesneriaceae: Evidence from DNA Sequences of the Chloroplast Gene *ndhF*

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TRIBAL RELATIONSHIPS IN
THE GESNERIACEAE:
EVIDENCE FROM DNA
SEQUENCES OF THE
CHLOROPLAST GENE *ndhF*¹

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ABSTRACT

The tribal relationships of the Gesneriaceae are investigated using *ndhF* sequences. A full analysis of 70 taxa including 16 species from the Scrophulariaceae, Bignoniaceae, and Acanthaceae as outgroups, resulted in two most-parsimonious trees of 5610 steps each. In all trees the Gesneriaceae were a monophyletic group and *Paulownia* was the closest single-species outgroup for the analysis. Further analyses eliminated all but the members of the Gesneriaceae and *Paulownia* in order to better assess relationships within the family. The smaller analysis resulted in a single most-parsimonious tree of 4613 steps. The Klugieae are identified as the sister to the remainder of the family and could potentially be separated as a distinct subfamily. The subfamilies Cyrtandroideae (excluding Klugieae) and Gesnerioideae are monophyletic. The placement of Coronanthereae in Cyrtandroideae does not have support from this analysis, whereas its placement in Gesnerioideae is supported. Alternatively, Coronanthereae could be segregated as a separate subfamily but in order to avoid a paraphyletic Gesnerioideae would either include the Napeantheae and Beslerieae or elevate these two tribes to an additional subfamily. Within Gesnerioideae the genus *Sinningia* is removed from the tribe Gloxinieae into the Sinningieae, which also contains the recently combined species *Sinningia brasiliensis* (*Lietzia*), as well as *Paliavana* and *Vanhouttea*. The Episcieae, Gesnerieae, Napeantheae, and Beslerieae are identified as monophyletic groups, as are the remainder of the Gloxinieae with *Sinningia* sensu lato removed. Within Cyrtandroideae, several well-supported, monophyletic lineages within the large, heterogeneous tribe Didymocarpeae are identified, and with the current data the tribe Trichosporeae appears to be polyphyletic. The distribution of chromosome numbers, nodal anatomy, placental structure, and stem modification are examined based on these molecular trees.

Investigations of higher level cladistic relationships (generic, familial, and above) have recently drawn a great deal of attention (*Annals of the Missouri Botanical Garden* Vol. 80(3); Olmstead et al., 1992, 1993; Donoghue et al., 1992; Cantino, 1992; Judd et al., 1994). These analyses have provided tremendous insights toward our classification system and process of classification, frequently drawing attention to families that have been separated on the basis of primarily woody versus herbaceous taxa (Cantino, 1992; Judd et al., 1994) or tropical versus temperate (Judd et al., 1994). More recently an investigation of the Lamiales sensu lato has indicated that the largest family in this order, Scrophulariaceae, is unlikely to be a monophyletic group (Olmstead et al., 1992, 1993; Olmstead & Reeves, 1995). A thorough investigation of the Scrophulariaceae utilizing DNA sequences from both the *rbcL* and *ndhF* genes has indicated that the family is comprised of at least two monophyletic groups with

several genera not having any strict affinity to the Scrophulariaceae or other related families included in the analysis (Olmstead & Reeves, 1995). Likewise, Olmstead and Reeves (1995) found that several families traditionally segregated from the Scrophulariaceae are best included as members of one of the two major lineages (e.g., Plantaginaceae).

Although most members of the Lamiales s.l. are temperate, there are some primarily tropical groups (Gesneriaceae, Acanthaceae, Bignoniaceae). In order to better assess whether the division between these families represents another artificial segregation based on distribution (tropical vs. temperate) or woody versus herbaceous (e.g., Bignoniaceae vs. Gesneriaceae), a thorough investigation of the Gesneriaceae was deemed necessary to complement the investigations that have already demonstrated monophyly of Acanthaceae (Scotland et al., 1995) and Bignoniaceae (R. Olmstead, pers. comm.), but have not sampled widely in the Gesneriaceae.

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The Gesneriaceae are a mid-sized to large plant family comprising 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). Given the diverse habits of the Gesneriaceae, it is not surprising that there is a wide array of morphological variation within the family. Corolla tubes may be long and prominent as in *Columnnea* L., or short as in *Saintpaulia* Wendl. Leaves are opposite in the majority of the family, but anisophylly, leading to an alternate arrangement with abscission of the smaller leaf, is common. Many of these morphologically diverse features of the Gesneriaceae are hypothesized as adaptations to the epiphytic habit (Ackerman, 1986).

The Gesneriaceae are a member of the Lamiales s.l. and are distinguished from other families in the order by the combination of five-lobed corollas, parietal placentation, and presence of endosperm in most taxa (Cronquist, 1981). However, because many of these characters vary within some members of the Gesneriaceae (including variation within individuals of some species), there has been considerable confusion regarding the placement of some genera. For example, members with axile placentation can be classified incorrectly with the Scrophulariaceae, and those genera lacking endosperm potentially may be classified with the Acanthaceae and Bignoniaceae.

There have been relatively few cladistic analyses performed within the Gesneriaceae (Kvist, 1990; Crisci et al., 1991; Boggan, 1991; Smith & Sytsma, 1994a, b, c; Smith, 1996), and only one (Smith, 1996) performed at the tribal level. A cladistic analysis is desirable to help resolve relationships, to determine if the family is monophyletic, and to improve classification within the family by rearranging tribes and subfamilies to reflect phylogenetic relationships.

Classifications of the Gesneriaceae traditionally recognize two subfamilies (Gesnerioideae and Cyrtandroideae) (Bentham, 1876; Burt, 1962, 1977; Fritsch, 1893, 1894), but others have included an additional subfamily (Coronantheroideae: Wiehler, 1983; Episcioideae: Ivanina, 1965). The division of the family is largely based on the uniform (Gesnerioideae), or uneven (Cyrtandroideae) enlargement of the cotyledons after germination (Burt,

1962). Another character that has been useful in separating the subfamilies is the presence (Gesnerioideae) or absence (Cyrtandroideae) of endosperm in the seed. In addition, the Gesnerioideae have a neotropical distribution and most species have inferior or semi-inferior ovaries, whereas the Cyrtandroideae are primarily paleotropical with superior ovaries. However, the geographic distribution and ovary position are not consistent within the subfamilies. Therefore, although the Cyrtandroideae can be defined by a synapomorphic character (uneven cotyledon development), the Gesnerioideae have been characterized by a symplesiomorphic character common to dicotyledons in general.

The two subfamilies have been divided further into 9–17 tribes (Bentham, 1876; Burt, 1962, 1977; Fritsch, 1893, 1894; Ivanina, 1965; Wiehler, 1983; Burt & Wiehler, 1995). The classification schemes differ due to the characters emphasized. For example, Fritsch (1893, 1894) placed the Columnneae in the Cyrtandroideae based on their superior ovary. Later, the Columnneae were moved to the Gesnerioideae due to the presence of uniform cotyledons (Burt, 1962, 1977) and combined into the Episcieae based on nodal anatomy (Wiehler, 1983). This paper presents a cladistic analysis of DNA sequences in order that phylogenetic relationships among taxa may be more clearly resolved, and a more stable classification scheme proposed.

The gene *ndhF* is a chloroplast gene that 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*. The reasons for the increased number of characters are that the gene is approximately 50% longer than *rbcL* (2103 vs. 1431 bp in tobacco [Wolfe, 1991]) and has a nucleotide substitution rate that is approximately two times higher than *rbcL* based on comparisons of rice and tobacco (Sugiura, 1989). In recent studies using this gene in the Acanthaceae, Scotland et al. (1995) found three times the number of characters compared to *rbcL*, and Olmstead and Sweere (1994) discovered 60% more variable characters with *ndhF* in the Solanaceae. Likewise, Clark et al. (1995) have found that *ndhF* sequences are informative for resolving relationships within the Poaceae, and Olmstead and Reeves (1995) have resolved several clades in a polyphyletic Scrophulariaceae. 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 As-

Table 1. Species sequenced in this study with Genbank submission numbers and voucher specimens. JFS - James F. Smith, WLW - Warren L. Wagner, DEB - Dennis E. Breedlove, SI - Smithsonian Institution, LG - Longwood Gardens. Letters in parentheses indicate herbarium where vouchers are deposited.

Species	Voucher	Genbank number
<i>Achimenes skinneri</i> Lindl.	SI 94-606	U62177
<i>Aeschynanthus micranthus</i> C. B. Clarke	JFS 643 (WIS)	U62169
<i>Agalmyla parasitica</i> (Lam.) Kuntze	SI 94-570	U62171
<i>Alloplectus meridensis</i> Klotzsch	JFS 1182 (WIS)	U62158
<i>Anna mollifolia</i> (W. T. Wang) W. T. Wang & K. Y. Pan	Skog 94-498	U62188
<i>Asteranthera ovata</i> (Cav.) Hanst.	Stewart 12234 (SRP)	U62204
<i>Besleria affinis</i> Morton	LG870575	U62162
<i>Boea hygroskopica</i> F. Muell.	SI 89-041	U62205
<i>Chirita sinensis</i> Lindl.	SI 94-111	U62189
<i>Codonanthe elegans</i> Wiehler	SI 82-45	U62178
<i>Columnnea schiedeana</i> Schlecht.	JFS 288 (WIS)	U62164
<i>Cyrtandra hawaiiensis</i> C. B. Clarke	WLW 6753 (BISH)	U62172
<i>Cyrtandra umbellifera</i> Merr.	WLW 6701 (BISH)	U62165
<i>Cyrtandromoea acuminata</i> Benth. & Hook.	JFS 3539 (SRP)	U62173
<i>Diastema racemiferum</i> Benth.	SI 85-98	U62156
<i>Didissandra frutescens</i> Clarke	SI 94-512	U62190
<i>Didymocarpus albomarginata</i> Hemsl.	SI 94-509	U62207
<i>Drymonia stenophylla</i> (J. D. Smith) H. E. Moore	JFS 2248 (WIS)	U62159
<i>Fieldia australis</i> Cunn.	Stewart s.n. (SRP)	U62196
<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> (HBK) Kunth	Dunn 9012051 (SRP)	U62157
<i>Hemiboea henryi</i> C. B. Clarke	SI 85-157	U62180
<i>Kohleria spicata</i> (Kunth) Oerst.	SI 94-552	U62181
<i>Lysionotus pauciflorus</i> Maxim.	SI 94-158	U62182
<i>Mitraria coccinea</i> Cav.	Stewart s.n. (SRP)	U62193
<i>Monophyllaea hirticalyx</i> Franch.	no voucher	U62168
<i>Monopyle macrocarpa</i> Benth.	no voucher	U62197
<i>Napeanthus costaricensis</i> Wiehler	no voucher	U62198
<i>Napeanthus macrostoma</i> Leeuwenberg	Feuillet (US)	U62161
<i>Negria rhabdothamnoides</i> F. Muell.	Nordenstam 8608 (S)	U62195
<i>Nematanthus hirsutus</i> (Mart.) Wiehler	Olmstead & Reeves, 1995	L36404
<i>Niphaea oblonga</i> Lindl.	SI 78-354	U62160
<i>Opithandra primuloides</i> (Miq.) B. L. Burt	SI 93-073	U62183
<i>Ornithoboea wildeana</i> Craib.	SI 93-075	U62166
<i>Paliavana prasinata</i> (Ker-Gawl.) Fritsch	SI 78-368	U62174
<i>Paraboea rufescens</i> (Franch.) Burt	Skog s.n. (US)	U62206
<i>Petrocosmea flaccida</i> Craib	SI 85-196	U62184
<i>Primulina tabacum</i> Hance	SI 93-040	U62167
<i>Ramonda myconi</i> (L.) Rehb.	Katzenstein s.n. (SRP)	U62185
<i>Rhynchoglossum notonianum</i> (Wall.) B. L. Burt	SI 94-378	U62179
<i>Rytidophyllum tomentosum</i> (L.) Mart.	SI 77-235	U62200
<i>Rytidophyllum auriculatum</i> Hook.	SI 94-524	U62199
<i>Saintpaulia rupicola</i> B. L. Burt	SI 94-492	U62176
<i>Sarmienta repens</i> Ruiz & Pavón	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>Solenophora obliqua</i> D. L. Denham & D. N. Gibson	DEB 71542 (CAS)	U62202
<i>Streptocarpus holstii</i> Engl.	Olmstead & Reeves, 1995	L36415
<i>Streptocarpus saxorum</i> Engl.	JFS s.n. (WIS)	U62170
<i>Titanotrichum oldhamii</i> (Hemsl.) Soler.	SI 86-106	U62187

Table 1. Continued.

Species	Voucher	Genbank number
<i>Vanhouttea lanata</i> Fritsch	SI 94-516	U62203
Outgroups		
<i>Antirrhinum majus</i> L.	Olmstead & Reeves, 1995	L36392
<i>Brillantaisia lamium</i> Benth.	Scotland et al., 1995	U12654
<i>Catalpa</i> sp.	Olmstead & Reeves, 1995	L36397
<i>Celsia arcturus</i> Jacq.	Olmstead & Reeves, 1995	L36398
<i>Crabbea reticulata</i> C. B. Clarke	Scotland et al., 1995	U12655
<i>Crossandra nilotica</i> Oliv.	Scotland et al., 1995	U12656
<i>Digitalis grandiflora</i> Mill.	Olmstead & Reeves, 1995	L36399
<i>Hygrophila corymbosa</i> Lindau	Scotland et al., 1995	U12661
<i>Martinella obovata</i> (HBK) Bureau & K. Schum.	Olmstead & Reeves, 1995	L36402
<i>Paulownia tomentosa</i> Steud.	Olmstead & Reeves, 1995	L36406
<i>Selago thunbergii</i> Choisy	Olmstead & Reeves, 1995	L36412
<i>Schlegelia parviflora</i> (Oerst.) Monachino	Olmstead & Reeves, 1995	L36410
<i>Scrophularia</i> sp.	Olmstead & Reeves, 1995	L36411
<i>Tabebuia heterophylla</i> (A. de Candolle) Britton	Olmstead & Reeves, 1995	L36416
<i>Verbascum thapsus</i> L.	Olmstead & Reeves, 1995	L36417
<i>Veronica catenata</i> Pennell	Olmstead & Reeves, 1995	L36419

teridae 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 1–1350, and 972–2044) from genomic DNA isolated from fresh, frozen, or silica gel dried material (Smith et al., 1992). Once amplification products were obtained, the sample was purified using PCR wizard purification preps (Promega) according to the manufacturer's instructions. The purified DNA then was subjected to cycle sequencing using the Silver Sequence method (Promega). This sequencing method resulted in sequences that could be read within six to eight hours after amplification was completed. The products of one round of amplification provided sufficient material for completing the gene sequences described here. The advantages of the silver staining procedure over radioactive methods are safety, minimal waste disposal, and speed.

CHOICE OF TAXA

The focus of this analysis was on the tribal relationships of the Gesneriaceae and comparison of the results of this analysis with one based on morphological data (Smith, 1996). Genera were selected to represent current and previous tribal classi-

fications within the family and, whenever possible, to match genera used in the morphological analysis. In some instances, a genus that had been used with the morphological analysis (Smith, 1996) was not readily available for the molecular analysis. Therefore this molecular analysis contains many taxa that have not been included in the morphological analysis, and direct comparisons will be made with a reduced data set at a future date. The species used in the analysis, voucher information, and Genbank accession numbers are included in Table 1.

Genera have been selected to represent the most recent tribal classifications with two to ten genera from each tribe (Tables 2 and 3). In order to represent current classification systems along with earlier systems, 48 genera were selected (Tables 2 and 3).

OUTGROUP SELECTION

Outgroups were selected to root the tree representing tribal relationships within Gesneriaceae. The best method for doing this is by outgroup comparison (Donoghue & Cantino, 1984; Maddison et al., 1984). The most appropriate outgroup for the tribes of the Gesneriaceae should be the most closely related plant family or clade. The Gesneriaceae have been placed in the order Lamiales s.l. in the subclass Asteridae (or equivalent groups of families) in numerous taxonomic treatments (Dahlgren, 1975; Thorne, 1976, 1983, 1992; Heywood, 1978; Takhtajan, 1980; Cronquist, 1981). However,

Table 2. Genera of Gesnerioideae (Burt & Wiehler, 1995) used and their classification status. NT = not treated. Subfamilial names are underlined to readily distinguish them from tribal names. Subtribal names are abbreviated as Colum. - Columneinae, Codon. - Codonanthinae.

Genus	Wiehler, 1983	Ivanina, 1965	Fritsch, 1893-94
	<u>Gesnerioideae</u>	<u>Gesnerioideae</u>	<u>Gesnerioideae</u>
<i>Achimenes</i>	Gloxinieae	Gloxinieae	Gloxinieae
<i>Gloxinia</i>	Gloxinieae	Gloxinieae	Gloxinieae
<i>Monopyle</i>	Gloxinieae	Bellonieae	Bellonieae
<i>Niphaea</i>	Gloxinieae	Bellonieae	Bellonieae
<i>Kohleria</i>	Gloxinieae	Kohlerieae	Kohlerieae
<i>Diastema</i>	Gloxinieae	Kohlerieae	Kohlerieae
<i>Sinningia</i>	Gloxinieae	Kohlerieae	Sinningieae
<i>Vanhouttea</i>	Gloxinieae	Kohlerieae	Kohlerieae
<i>Paliavana</i>	Gloxinieae	Reichsteinerieae	Kohlerieae
<i>Lietzia</i>	Gloxinieae	Reichsteinerieae	Sinningieae
<i>Solenophora</i>	Gloxinieae	Solenophoreae	Solenophoreae
<i>Gesneria</i>	Gloxinieae	Gesnerieae	Gesnerieae
<i>Rytidophyllum</i>	= <u>Gesneria</u>	Gesnerieae	Gesnerieae
		<u>Episcioideae</u>	<u>Cyrtandroideae</u>
<i>Columnea</i>	Episcieae	Columneae	Columneae-Colum.
<i>Codonanthe</i>	Episcieae	Columneae	Columneae-Codon.
<i>Nematanthus</i>	Episcieae	Columneae	Columneae-Colum.
<i>Alloplectus</i>	Episcieae	Episcieae	Columneae-Colum.
<i>Drymonia</i>	Episcieae	Episcieae	Columneae-Colum.
<i>Besleria</i>	Beslerieae	Episcieae	Beslerieae
<i>Gasteranthus</i>	Beslerieae	NT	NT
<i>Napeanthus</i>	Napeantheae	Episcieae	Klugieae
	<u>Coronantheroideae</u>	<u>Cyrtandroideae</u>	
<i>Asteranthera</i>	Coronanthereae	Mitrarieae	Coronanthereae
<i>Sarmienta</i>	Coronanthereae	Mitrarieae	Coronanthereae
<i>Mitraria</i>	Coronanthereae	Mitrarieae	Coronanthereae
<i>Fieldia</i>	Coronanthereae	Mitrarieae	Coronanthereae
<i>Negria</i>	Coronanthereae	Coronanthereae	Coronanthereae

the relationships among these families are somewhat ambiguous. A recent cladistic analysis of these families based on DNA sequencing of the chloroplast encoded *rbcL* gene resulted in poor resolution of the relationships of these families (Olmstead et al., 1993), although these relationships have been more resolved with the addition of *ndhF* sequences (Olmstead & Reeves, 1995).

Three families from the Lamiales s.l. were used as outgroups for this analysis. These were the Acanthaceae, Bignoniaceae, and Scrophulariaceae. Sequences for sixteen species of these three families were obtained via Genbank (Table 1; Olmstead & Reeves, 1995; Scotland et al., 1995) and included representatives from three lineages identified within the Scrophulariaceae (Olmstead & Reeves, 1995). Initial analyses used all 16 species as the outgroup. Subsequent analyses used only Gesneriaceae with *Paulownia* Sieb. & Zucc. as the outgroup.

PHYLOGENETIC ANALYSIS

Phylogenetic divergence was reconstructed using PAUP version 3.1.1 (Swofford, 1993) to implement Wagner parsimony (Farris, 1970; Farris et al., 1970; Swofford & Maddison, 1987). This program allows parallelisms and reversals (homoplasy), and provides an option for missing data. 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 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 (Mad-

Table 3. Genera of Cyrtandroideae (Burt & Wiehler, 1995) used and their classification status. NT = not treated. The tribe, Didymocarpeae, is abbreviated Didy. in order to show the subtribal classification system of Ivanina (1965) and Fritsch (1893, 1894).

Genus	Burt, 1962,77	Ivanina, 1965	Fritsch, 1893-94
<i>Ramonda</i>	Didymocarpeae	Ramondeae	Ramondeae
<i>Saintpaulia</i>	Didymocarpeae	Saintpaulieae	Ramondeae
<i>Opithandra</i>	Didymocarpeae	Didy.-Roettlerineae	NT
<i>Didymocarpus</i>	Didymocarpeae	Didy.-Roettlerineae	Didy.-Roettlerineae
<i>Didissandra</i>	Didymocarpeae	Didy.-Roettlerineae	Didy.-Orecharineae
<i>Anna</i>	Didymocarpeae	Didy.-Roettlerineae	NT
<i>Chirita</i>	Didymocarpeae	Didy.-Roettlerineae	Didy.-Roettlerineae
<i>Petrocosmea</i>	Didymocarpeae	Didy.-Roettlerineae	Ramondeae
<i>Titanotrichum</i>	anomalous	NT	NT
<i>Cyrtandromoea</i>	Loxonieae/Scroph.	Klugieae	Beslerieae
<i>Paraboea</i>	Didymocarpeae	Didy.-Roettlerineae	Didy.-Roettlerineae
<i>Boea</i>	Didymocarpeae	NT	Streptocarpeae
<i>Hemiboea</i>	Didymocarpeae	Didy.-Roettlerineae	NT
<i>Primulina</i>	Didymocarpeae	Didy.-Roettlerineae	Klugieae
<i>Streptocarpus</i>	Didymocarpeae	Didy.-Streptocarp.	Streptocarpeae
<i>Ornithoboea</i>	Didymocarpeae	Didy.-Streptocarp.	Streptocarpeae
<i>Aeschynanthus</i>	Trichosporeae	Trichosporeae	Trichosporeae
<i>Agalmyla</i>	Trichosporeae	Trichosporeae	Trichosporeae
<i>Lysionotus</i>	Trichosporeae	Trichosporeae	Trichosporeae
<i>Cyrtandra</i>	Cyrtandreae	Cyrtandreae	Cyrtandreae
<i>Rhynchoglossum</i>	Klugieae	Klugieae	Klugieae
<i>Monophyllaea</i>	Klugieae	Klugieae	Beslerieae

dison, 1991). Therefore, it is important to repeat the analysis several times. To do this, the search strategy of Olmstead and Palmer (1994) was implemented: searching for 1000 trees each 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." This strategy was used in the full analysis with all 16 non-Gesneriaceae taxa designated as outgroups. Likewise, the same strategy was used with only the members of the Gesneriaceae and *Paulownia* as the outgroup, and with constraints options.

Branch support analysis was performed to examine trees that were six or fewer steps longer than the most-parsimonious tree (Bremer, 1988; Donoghue et al., 1992; Bremer, 1994). 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 six steps longer with the filter option of PAUP.

The *ndhF* sequences used here had several six to twelve base pair insertions or deletions (indels)

inferred from gaps in the sequence alignments, which in previous analyses had been re-scored as binary characters and used as either an independent data set or combined with the sequence data (Scotland et al., 1995). These indels were viewed as having phylogenetic importance (Scotland et al., 1995); therefore indels found in the Gesneriaceae were removed and examined independently of sequence data for their phylogenetic utility.

The monophyly of various tribal relationships not obtained in the most-parsimonious trees was examined by using the constraints option of PAUP. These included the Trichosporeae, the Didymocarpeae, the inclusion of Klugieae in Cyrtandroideae, and Sinningieae in Gloxinieae. Also, since the analysis with all 16 outgroup taxa resulted in the placement of *Nematanthus* Schrader and Klugieae in discrepant positions from traditional classifications, an analysis with all 16 outgroups constrained *Nematanthus* to the Gesnerioideae, and the Klugieae from the Gesnerioideae. The position of Klugieae and *Nematanthus* was also examined by constructing a user-defined tree with a topology of one of the two most-parsimonious trees except that *Nematanthus* was placed in the Episcieae, and Klugieae was placed as sister to the remainder of the Gesneriaceae. This user-defined tree was then the

starting tree for a search using TBR and mulpars "on."

RESULTS

Complete sequences for the *ndhF* gene were obtained for 52 species of Gesneriaceae (Table 1). These sequences were supplemented with sequences from an additional 18 species (2 within Gesneriaceae and 16 from related families) from Genbank (Table 1). The complete sequences resulted in 849 phylogenetically informative characters among all 70 species in the full analysis. A smaller analysis focused on only the Gesneriaceae species with *Paulownia* as the outgroup. Within this smaller analysis 690 nucleotide positions were found to be phylogenetically informative. Indels were found at several positions in the Gesneriaceae from the sequences used in this analysis. Two widespread insertions were a 12 bp insertion at position 1440 and a 6 bp insertion at 1548. Other insertions were autapomorphic for species or genera used in the analysis (unpublished results). No insertions were used in the analysis. The 6 bp insertion was symplesiomorphic for the Gesneriaceae. The 12 bp insertion was also symplesiomorphic for the Gesneriaceae; however, sequence divergence within this insertion provides an additional synapomorphy for the clade comprised of *Columnea*, *Drymonia* Mart., and *Alloplectus* Mart. (Fig. 4), where a single base pair transition characterizes these three genera. Other base pair substitutions and insertions were found within this 12 bp insertion but, with the current level of sampling, were autapomorphic.

Cladistic analysis was performed initially with all 70 taxa of the four families (Gesneriaceae, Scrophulariaceae, Acanthaceae, and Bignoniaceae) and all taxa in the three outgroup families designated as the outgroup. This analysis resulted in two trees of 5610 steps each (consistency index (CI) = 0.30, retention index (RI) = 0.48), all of which indicated the Gesneriaceae were a monophyletic family and that the genus *Paulownia* (Scrophulariaceae) was the closest outgroup (Figs. 1, 2).

Subsequent analyses were performed to minimize computer analysis time that utilized only the Gesneriaceae and *Paulownia* as a designated outgroup. This reduced analysis resulted in a single most-parsimonious tree of 4613 steps (CI = 0.27, RI = 0.38) (Figs. 3, 4). Some taxa that have been thought to be monophyletic, or comprised tribes, were examined using the constraints option of PAUP to determine the impact of the monophyletic grouping on the remainder of the data and to determine the number of additional steps required to

construct these trees. The analysis required four additional steps to create a monophyletic Trichosporeae, five for a monophyletic Didymocarpeae, two to include the Klugieae in the Cyrtandroideae, and four to include the Sinningieae in the Gloxinieae. Constraining the analysis of all 70 taxa to place Klugieae as the sister to the Gesneriaceae and *Nematanthus* within the Episcieae resulted in four trees 58 steps longer than the most-parsimonious trees regardless of whether the constraint option of PAUP, or user defined trees were implemented.

DISCUSSION

The cladistic analysis of 54 species of Gesneriaceae with 16 species of Scrophulariaceae, Bignoniaceae, and Acanthaceae as outgroups resulted in a monophyletic Gesneriaceae with the single genus *Paulownia* (Scrophulariaceae) indicated as the closest outgroup (Figs. 1, 2). These results verified that the Gesneriaceae are distinct from other members of the Lamiales s.l. and not an artificial unit based on their largely tropical distribution and herbaceous habit as has been seen for some family pairs (Judd et al., 1994). The full analysis is largely in agreement with the position of the taxa in the reduced analysis with the exception of the positions of *Nematanthus* and the tribe Klugieae. The placement of *Nematanthus* as the sister to the remainder of the family is very far removed from its traditional classification within the Episcieae (Fig. 1). Likewise the Klugieae are placed unusually in the subfamily Gesnerioideae (Fig. 2). The most likely explanation for the anomalous placement of these taxa is the high level of homoplasy between the Gesneriaceae and the outgroups. This is exemplified when 15 of the 16 outgroup species are removed from the analysis. In the reduced analysis both *Nematanthus* and Klugieae are in more expected positions regarding relationships to the remainder of the family. An alternative explanation is that because of the size of the data set, PAUP did not find the shortest tree and that a shorter tree with all 70 species exists that places *Nematanthus* and the Klugieae in their more expected relationships. This latter explanation is unlikely since searches constraining these taxa to their more traditional positions, or a user-defined tree that placed them there, resulted in four trees that were 58 steps longer.

The reduced analysis resulted in a single most-parsimonious tree (Figs. 3, 4). Three major monophyletic divisions within the family correspond to subfamilies Gesnerioideae and Cyrtandroideae (minus tribe Klugieae) and tribe Klugieae in a separate position as a potential third subfamily. Traditional

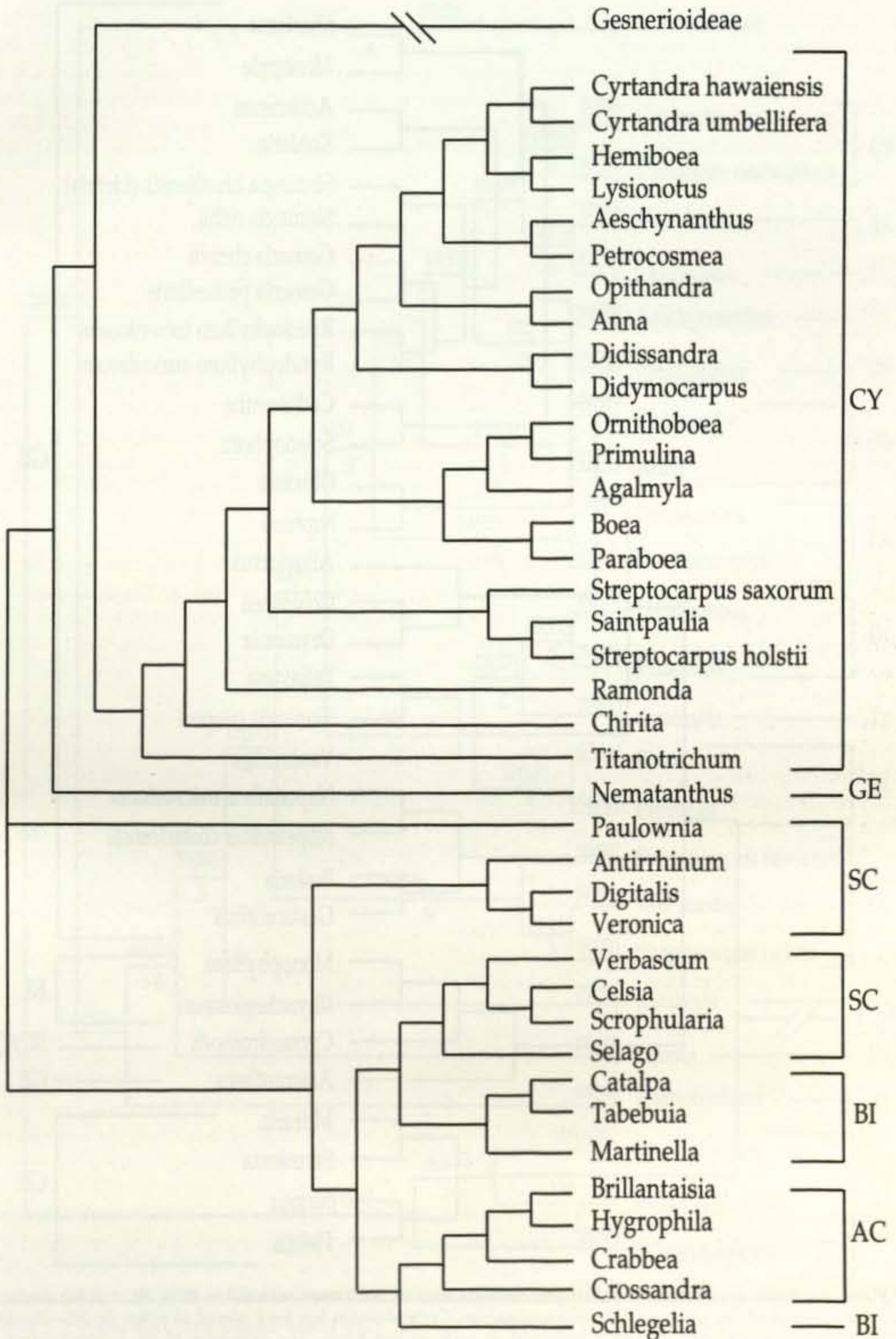


Figure 1. Strict consensus of two most-parsimonious trees of 5610 steps each (CI = 0.30, RI = 0.48) displaying the outgroup taxa, BI—Bignoniaceae, AC—Acanthaceae, SC—Scrophulariaceae, and the subfamilies of the Gesneriaceae, GE—Gesnerioideae and CY—Cyrtandroideae. The remainder of the Gesnerioideae are displayed in Figure 2. See text for explanation of position of *Nematanthus* in this cladogram.

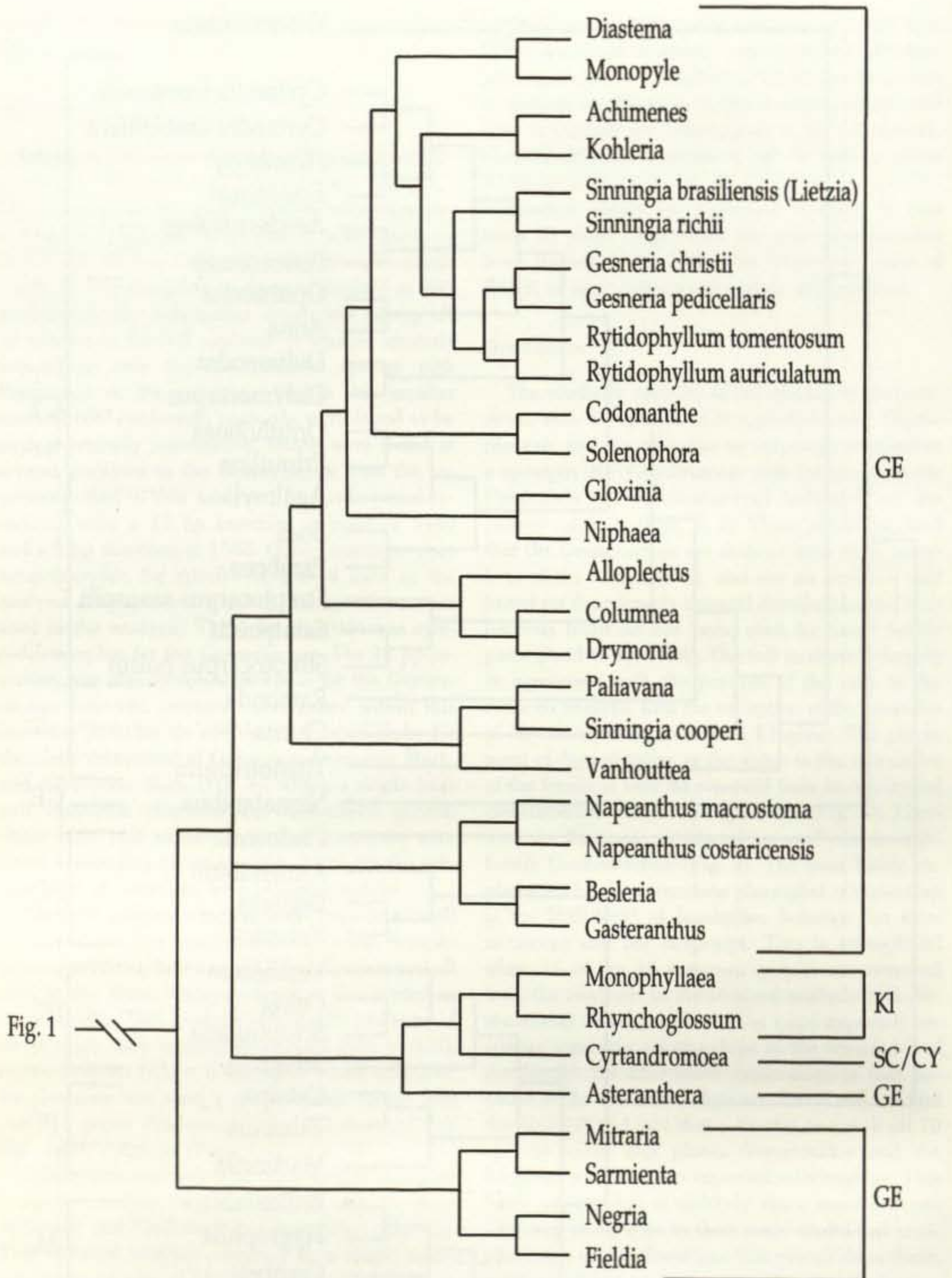


Figure 2. Strict consensus of two most-parsimonious trees of 5610 steps each ($CI = 0.30$, $RI = 0.48$) displaying the GE—Gesnerioideae, and some CY—Cyrtandroideae. *Cyrtandromoea* has been placed in either the SC—Scrophulariaceae or the Cyrtandroideae and is indicated as such on this figure. The remainder of the Cyrtandroideae are displayed in Figure 1. See text for the explanation of position of the tribe Klugieae (Kl) in this cladogram.

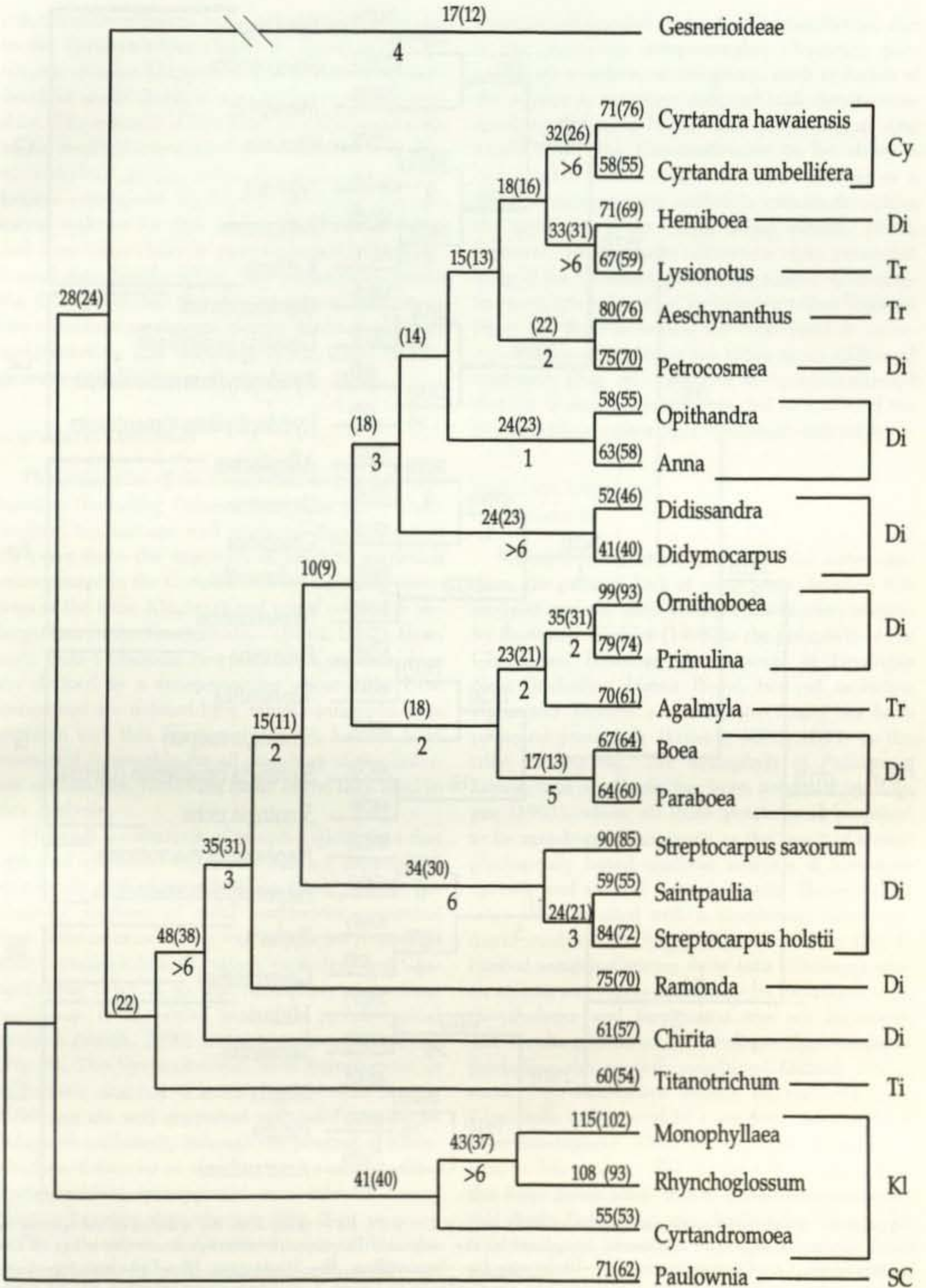


Figure 3. Single most-parsimonious tree of 4613 steps (CI = 0.29, RI = 0.38) from the analysis of the species in the Gesneriaceae with only *Paulownia* (SC—Scrophulariaceae) designated as the outgroup. Displayed in this figure are the tribes of the Cyrtandroideae, Kl—Klugieae, Ti—Titanotricheae, Di—Didymocarpeae, Tr—Trichosporeae, and Cy—Cyrtandreae. The Gesnerioideae are displayed in Figure 4. Numbers along branches are the synapomorphies that support those clades. Numbers in parentheses indicate those synapomorphies that are homoplasy in this tree. Numbers below branches are decay values. Branches with no value indicated have a decay value of 1.

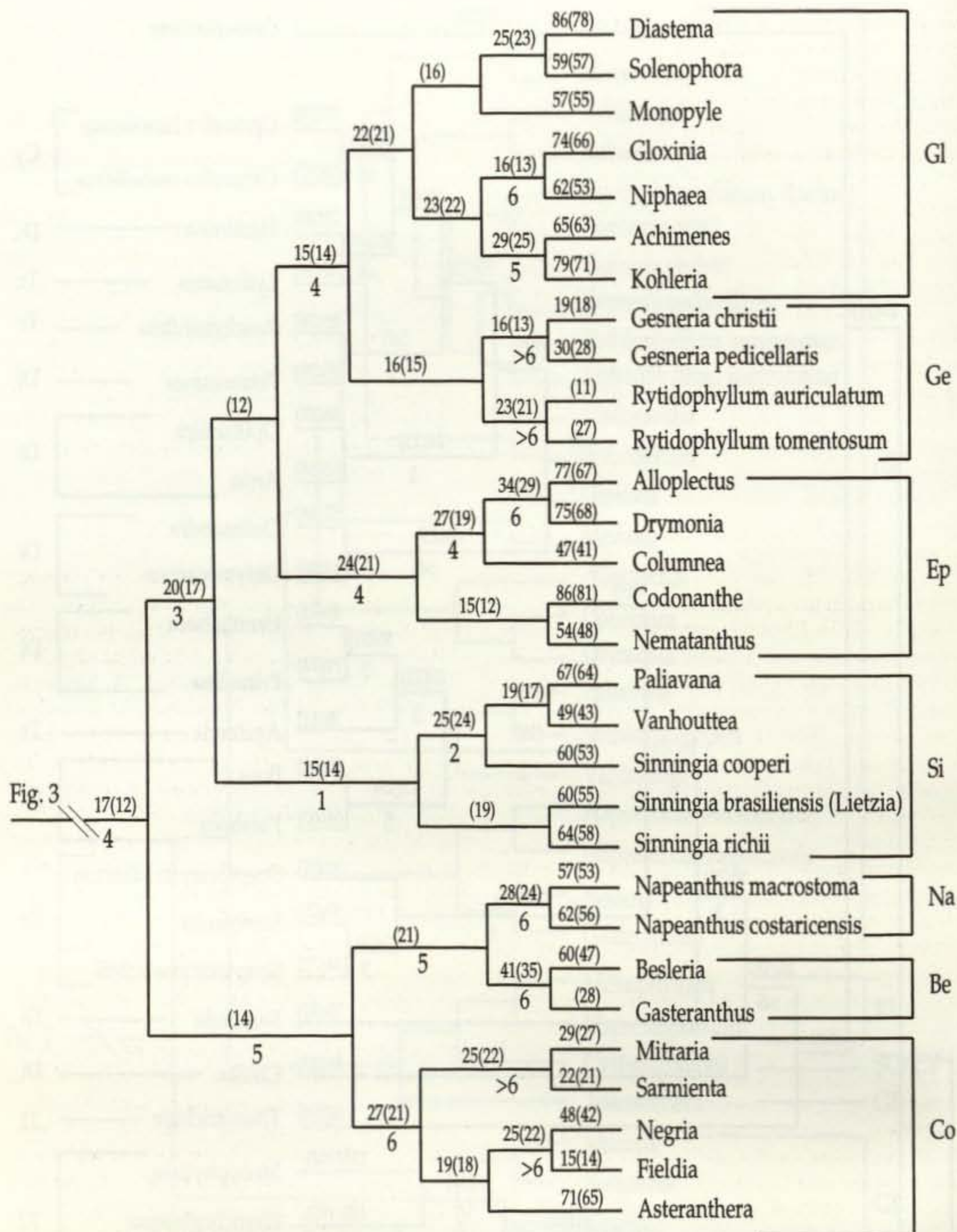


Figure 4. Single most-parsimonious tree of 4613 steps (CI = 0.29, RI = 0.38) from the analysis of the species in the Gesneriaceae with only *Paulownia* designated as the outgroup. Displayed in this figure are the tribes of the Gesnerioideae, Co—Coronanthereae, Be—Beslerieae, Na—Napeantheae, Si—Sinningieae, Ep—Episcieae, Ge—Gesnerieae, and Gl—Gloxinieae. The Cyrtandroideae are displayed in Figure 3. Numbers along branches are the synapomorphies that support those clades. Numbers in parentheses indicate those synapomorphies that are homoplastic in this tree. Numbers below branches are decay values. Branches with no value indicated have a decay value of 1.

classification schemes have placed tribe Klugieae in the Cyrtandroideae (Table 3); however, the inclusion of tribe Klugieae within subfamily Cyrtandroideae would result in a paraphyletic Cyrtandroideae. The removal of this tribe to a third subfamily would result in a monophyletic Cyrtandroideae. The monophyletic groups within the subfamily Gesnerioideae correspond highly with traditional classification systems for this subfamily (Wiehler, 1983) and a previous cladistic analysis based on morphological data (Smith, 1996). The relationships within the Cyrtandroideae are less congruent with previous taxonomic treatments, mainly due to the limited understanding and sampling of the large, heterogeneous tribe Didymocarpeae (Burt, 1962).

SUBFAMILIAL GROUPINGS

The separation of the Gesneriaceae into two subfamilies (including Coronanthereae in the Gesnerioideae) has become well accepted during the past 30 years since the discovery of unequal cotyledon enlargement in the Cyrtandroideae (including members of the tribe Klugieae) and equal cotyledon enlargement in the Gesnerioideae (Burt, 1962). However, from a cladistic viewpoint the Cyrtandroideae are defined by a synapomorphy whereas the Gesnerioideae are defined by a symplesiomorphy. One problem with this character is that it has not been examined thoroughly for all members of the different subfamilies, including many of the taxa used in this analysis.

Although an analysis of morphological data that included cotyledon expansion did not support the monophyly of the Cyrtandroideae (Smith, 1996), the cladistic analysis of *ndhF* sequences presented here demonstrates both a well-supported monophyletic Cyrtandroideae (Klugieae excluded) and Gesnerioideae (Figs. 3, 4). The monophyly of the Gesnerioideae is supported in both a morphological analysis (Smith, 1996) and this molecular analysis (Fig. 4). The Cyrtandroideae were paraphyletic in a cladistic analysis of morphological data (Smith, 1996) but are well supported with *ndhF* sequences (Klugieae excluded), although the position of *Titanotrichum* Solereder as sister to the remainder of the Cyrtandroideae is supported with only 22 homoplastic character state changes (Fig. 3).

The placement of Coronanthereae within the Gesnerioideae is well supported with *ndhF* sequences (Fig. 4) as it is with morphological data (Smith, 1996). This tribe does not belong in the subfamily Cyrtandroideae as had been proposed earlier (Fritsch, 1894). Wiehler (1983) in his treatment of the neotropical Gesneriaceae suggested a

separate subfamilial status for Coronanthereae due to the numerous autapomorphic characters possessed by members of this group, such as fusion of the nectary to the ovary wall and high chromosome numbers (Wiehler, 1983). The morphological data would allow the Coronanthereae to be either a monophyletic tribe within the Gesnerioideae or a separate monophyletic subfamily without disrupting the taxonomy of any other group (Smith, 1996). However, based on the molecular data presented here, if the Coronanthereae were raised to subfamily level, it would either include the tribes Napeantheae and Beslerieae from the Gesnerioideae or necessitate elevating these two tribes as an additional subfamily (Fig. 4). Therefore it is recommended that the Coronanthereae be treated as a tribe of the Gesnerioideae rather than a separate subfamily.

TRIBAL RELATIONSHIPS

GESNERIOIDEAE

Among the relationships within the Gesnerioideae, the primary lack of congruence between this analysis and the most recent classification scheme by Burt and Wiehler (1995) is the polyphyly of the Gloxinieae. However, the removal of *Sinningia* Nees (including *Lietzia* Regel, but not including *Paliavana* Vandelli or *Vanhouttea* Lem.) has been proposed previously (Fritsch, 1893, 1894) as the tribe Sinningieae. The monophyly of *Paliavana*, *Lietzia*, and *Sinningia* has been proposed by Boggan (1991), where all three genera were proposed to be members of *Sinningia* as the result of a morphologically based cladistic analysis of *Sinningia* species and several related genera. These results were not supported with a morphology-based cladistic analysis (Smith, 1996), most likely due to limited sampling among these taxa (*Sinningia sensu stricto* was represented only by *Sinningia* sect. *Corytholoma* and *Vanhouttea* was not included). The results presented here indicate that *Sinningia* (including the recently combined *Lietzia*), *Paliavana*, and *Vanhouttea* should be removed from Gloxinieae and placed in a separate monophyletic tribe Sinningieae. Although *Sinningia* is paraphyletic in this analysis (Fig. 4), limited sampling from this large genus leads only to a tentative conclusion that both *Paliavana* and *Vanhouttea* should be combined into *Sinningia* to create a monophyletic genus.

The sister relationship of the Beslerieae and Napeantheae has been hinted at based on the overlap of several diagnostic characters between these tribes (Skog, 1995; Skog & de Jesus, 1996). However, the sister relationship of these two tribes to

the Coronanthereae (Fig. 4) has not been proposed previously. Although the morphological data did not indicate sister group status, the data did indicate a close affinity among these three tribes (Smith, 1996).

Among the recent classification schemes proposed for the Gesnerioideae, Burt and Wiehler's (1995) is the closest approximation to the results obtained in this study. The subdivision of Wiehler's (1983) Gloxinieae into the Bellonieae, Kohlerieae, Rechsteinerieae, and Solenophoreae (Ivanina, 1965) (Table 2) is not supported by this cladistic analysis. Likewise separating Wiehler's (1983) Gloxinieae into Bellonieae, Kohlerieae, and Solenophoreae (Table 2; Fritsch, 1893, 1894) is not supported except for the removal of the Sinningieae (Fig. 4), which would also necessarily include *Palaivana* and *Vanhouttea* (included in Fritsch's Kohlerieae; Table 2). The placement of *Napeanthus* G. Gardner in the Klugieae (Cyrtandroideae) (Table 2) as proposed by Fritsch (1893, 1894) is inappropriate.

CYRTANDROIDEAE

Burt's (1962, 1977) classification system for the Cyrtandroideae is closer in agreement to this cladistic analysis than previous classification schemes (Ivanina, 1965; Fritsch, 1893, 1894). However, the monophyly of the largest tribe, the Didymocarpeae, is not supported by this analysis (Fig. 3). Likewise none of the subtribes created by Ivanina (1965) or Fritsch (1893, 1894) are supported as monophyletic groups (Fig. 3, Table 3). The Trichosporeae are not supported as a monophyletic clade (Fig. 3). Although this tribe was well supported in the morphological analysis (Smith, 1996), four additional steps beyond the most-parsimonious tree are required to make this clade monophyletic with *ndhF* data.

The position of *Titanotrichum* has been problematic, although this genus has consistently remained in the Gesneriaceae (Burt, 1962, 1977; Wang et al., 1992; Burt & Wiehler, 1995). *Titanotrichum* is a member of the Cyrtandroideae based on these data, and perhaps may be viewed best as a monotypic tribe (Titanotricheae; Wang et al., 1992), sister to the remainder of the subfamily. However, the position of *Titanotrichum* as the sister to the remainder of the Cyrtandroideae is only weakly supported with 22 homoplastic character state changes, and the resolution of its placement is lost in the strict consensus of all trees only one step longer than the most-parsimonious tree. Therefore, it is likely that *Titanotrichum*, or the lineage leading to

this species, diverged early in the evolution of the family. The placement of *Titanotrichum* within the Gesneriaceae is discussed elsewhere (Smith et al., 1997).

The Didymocarpeae are a large heterogeneous tribe that includes the majority of genera in the Cyrtandroideae (Burt, 1962, 1977; Wang et al., 1992). In this analysis it is a paraphyletic assemblage that includes the Cyrtandreae and Trichosporeae (Figs. 1, 3). Because of the large size of the Didymocarpeae, and the limited sampling of the tribe in this analysis, no conclusions regarding its monophyly, or potential division into other tribes, are recommended at this time. Further morphological investigations in this tribe are under way (B. L. Burt and A. Weber, pers. comm.), and a cladistic analysis that focuses on this group will be valuable toward understanding its relationships. Several well-supported monophyletic groups within the Didymocarpeae can be identified (*Boea* Commerson ex Lamarck/*Paraboea* (C. B. Clarke) Ridley, *Hemiboea* C. B. Clarke/*Lysionotus* D. Don, *Didissandra* C. B. Clarke/*Didymocarpus* Wallich, and *Streptocarpus* Lindl./*Saintpaulia*). It should be noted that *Didissandra* and *Didymocarpus*, although forming a monophyletic clade in this analysis, are both large heterogeneous genera and that sampling different species may have resulted in different placement. By focusing on morphological characters of these groups it may be possible to identify more inclusive monophyletic tribes out of the paraphyletic Didymocarpeae. Much greater sampling within this large group will be necessary before any major realignment can begin.

An unexpected result of this analysis is the paraphyly of *Streptocarpus*. The most likely explanation for this paraphyly is limited sampling, with only two species of *Streptocarpus* and one of *Saintpaulia*. However, it is interesting to note that *Saintpaulia* is one of the few genera within the Gesneriaceae to have a chromosome number of $n = 15$ (Skog, 1984). The only other genera that share this number are some species of *Streptocarpus*, including both *S. saxorum* and *S. holstii*, and some species of *Aeschynanthus* Jack (Skog, 1984). The possibility that *Saintpaulia* is derived from within *Streptocarpus*, as indicated by *ndhF* sequences and chromosome numbers, currently is being investigated with greater sampling.

The Trichosporeae traditionally have been viewed as a monophyletic tribe defined by the presence of seed appendages not present elsewhere within the family (Burt, 1962, 1977; Wang et al., 1992). Based on morphological data, the Trichosporeae were one of the most strongly supported

tribes in a morphology-based cladistic analysis (Smith, 1996). However, it is apparent from this analysis of *ndhF* sequences that the selection of characters that define the Trichosporeae is inappropriate (e.g., seed appendages are common in the closely related Bignoniaceae). Alternatively, it is possible that inadequate sampling from the Trichosporeae or the large tribe Didymocarpeae may be causing the separate placement of the three genera sampled from the Trichosporeae. This latter hypothesis is unlikely, because one of the more strongly supported clades in the analysis placed *Lysionotus* (Trichosporeae) with *Hemiboaea* (Didymocarpeae) and away from the other genera of the Trichosporeae (Fig. 3).

KLUGIEAE

The Klugieae are monophyletic and are the sister group to the remainder of the Gesneriaceae (Fig. 3). The placement of this tribe in the Gesnerioideae (Fig. 2) in the full data analysis most likely is due to homoplasy or the result of an incomplete search for the shortest tree. The monophyly and sister group status of this tribe also was supported with a cladistic analysis of morphological data (Smith, 1996). The Klugieae possess numerous autapomorphic characters relative to other Gesneriaceae such as narrow medullary rays, and verrucate edges of the cells of the seed coat (Smith, 1996). The placement of *Cyrtandromoea* Zoll. in the Klugieae of the Cyrtandroideae was proposed previously by Ivanina (1965), although other investigations indicated that this genus should be excluded from the Gesneriaceae on the basis of floral anatomy (Burt, 1965; Singh & Jain, 1978). The placement of *Cyrtandromoea* in the Gesneriaceae is discussed elsewhere (Smith et al., 1997).

EVOLUTION OF NON-MOLECULAR CHARACTER STATES CHROMOSOME NUMBERS

Several chromosome counts are synapomorphic and non-homoplastic based on this cladistic analysis. Large numbers of chromosomes ($n = 30+$) are unique to the Coronanthereae and would serve as an additional character to separate this tribe from the remainder of the family (Skog, 1984). A chromosome base number, x , of 14 characterizes the Gesnerieae (Wiehler, 1983; Skog, 1984). The cladistic analysis of morphological data was unable to separate the Gesnerieae from the tribe Gloxinieae although it represented a monophyletic group within it (Smith, 1996). The inclusion of chromosome numbers (which were excluded due the large number of character states) might have removed Ges-

nerieae from Gloxinieae as seen here with sequence data.

Most Gloxinieae sampled here (excluding *Solenophora* Benth., *Niphaea* Lindl., and *Achimenes* Pers.) have $x = 13$ (Wiehler, 1983; Skog, 1984). In addition, the members of the Sinningieae that have been examined also have $x = 13$ (Skog, 1984). This similarity in chromosome base number, along with other character states, has led previous researchers to include the members of the Sinningieae within the Gloxinieae (Wiehler, 1983). However, based on the analysis presented here, the Sinningieae are best viewed as a tribe separated from the Gloxinieae, and $x = 13$ is homoplastic.

Other homoplastic chromosome numbers are $x = 11$ (*Niphaea* and *Achimenes*), and $x = 9$ (*Alloplectus*, *Drymonia*, *Columnea*, and some *Didymocarpus* species). Although most of these homoplastic counts serve little phylogenetic utility, the count of $x = 9$ serves to characterize a portion of the Episcieae. Most members of the Episcieae have $x = 9$, but taxa with $x = 8$ (*Codonanthe* (Mart.) Hanst. and *Nematanthus*) may represent another clade (Fig. 4). Further sampling within the Episcieae may reveal if this clade (Fig. 4) continues to be supported or is the result of sampling in this analysis.

Other chromosome counts in the Cyrtandroideae are highly variable even within genera, and no pattern emerges from the counts of the species that have been included in the analysis, with the exception of the *Streptocarpus/Saintpaulia* counts discussed above.

NODAL ANATOMY

Another useful character for the Gesneriaceae is nodal anatomy (Wiehler, 1983). Unfortunately only the subfamily Gesnerioideae has been sampled thoroughly for this character, and the lack of data for the Cyrtandroideae necessitated the exclusion of this character from the morphological analysis (Smith, 1996). However, if nodal anatomy is mapped onto the trees from this molecular analysis, this character can provide useful phylogenetic information. The tribe Episcieae (Fig. 4) is defined by a three-trace trilacunar node that is unique among the Gesnerioideae, although this character state is known from the Cyrtandroideae. The unique presence of this character state within the Gesnerioideae adds further support to the monophyly of the Episcieae. The three-trace trilacunar node may be symplesiomorphic for the Cyrtandroideae, as all taxa with available data for this character (*Saintpaulia*, *Streptocarpus*, and *Cyrtandra* Forster & Forster) possess a three-trace trilacunar node except

Aeschynanthus, which has a one-trace trilacunar node common to the Gesnerioideae.

PLACENTA

The placenta in the Gesneriaceae is either intact or divided to the base (Ivanina, 1965). This character was included in a cladistic analysis of morphological data and served as a character state that brought the Episcieae, Beslerieae, and Napeantheae together in a single clade (Smith, 1996) as the only taxa sampled that had divided placentae. Although this character state is consistent with the relationship between the Napeantheae and Beslerieae, the character state is homoplastic between the Episcieae and Napeantheae/Beslerieae based on the data presented here (Fig. 4).

STEM MODIFICATION

Several members of the Gesneriaceae possess modifications of the stems (rhizomes and tubers), presumably as adaptations to periodic dry seasons (Wiehler, 1983). The presence of scaly rhizomes is found almost exclusively, and is widespread, within the Gloxinieae (Wiehler, 1983). Among the taxa sampled here, the presence of scaly rhizomes serves as a synapomorphy for the tribe Gloxinieae, although they are not known from the woody genus *Solenophora*. Scaly rhizomes also are known from the Cyrtandroideae, including *Titanotrichum* (Kao & DeVol, 1972; Wang et al., 1992).

Tubers are widespread among species of *Sinningia* including *Lietzia*, which has recently been combined into *Sinningia* (Wiehler & Chautems, 1995). Although tubers serve to unite these genera, and to separate them from the Gloxinieae, tubers are not known from *Paliavana* or *Vanhouttea*. However, not all species of *Sinningia* are tuberous, and the lack of tubers in these species can be regarded as intra-tribal or intra-generic variation. Tubers also are known from several species in the Episcieae (*Drymonia*, *Chrysothemis* Dcne., *Nautilocalyx* Lind. ex Hanst., *Paradrymonia* Hanst., and *Rhoogeton* Leeuwenberg) as well as one member of the Gloxinieae (*Lembocarpus* Leeuwenberg). Further studies that include these taxa will hopefully resolve the number of times tubers have originated within the Gesneriaceae.

BIOGEOGRAPHY

The traditional division of the Gesneriaceae into two subfamilies (excluding the Klugieae, which may stand best as a third subfamily) is well supported in this analysis and is in agreement with the

biogeographic distribution of these taxa. The Cyrtandroideae (excluding the Klugieae) are distributed almost exclusively in the paleotropics with a few temperate European and Asian species. Two African genera were included in this analysis, both of which are in a single clade (*Saintpaulia* and *Streptocarpus*). Only one of the European taxa (*Ramonda* L. C. Richard) has been included in this analysis; therefore nothing can be inferred regarding the origin of these taxa at this time.

Members of the tribe Klugieae range from India to south China, Taiwan, the Philippines through Malaysia, Indonesia, and into New Guinea. Discrepancies from this distribution include a single species of *Rhynchoglossum* Blume found in Central and South America. The presence of *Rhynchoglossum azureum* (Schlecht.) B. L. Burt in the Neotropics represents a secondary dispersal event in the family, because all other members of the Klugieae are found in the Old World.

The Gesnerioideae are almost exclusively neotropical, but with the inclusion of the Coronantheae within this subfamily the Gesnerioideae now encompass several Australian and South Pacific island species.

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