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PHYLOGENETIC RESOLUTION WITHIN THE TRIBE EPISCIEAE (GESNERIACEAE): CONGRUENCE OF ITS AND *NDHF* SEQUENCES FROM PARSIMONY AND MAXIMUM-LIKELIHOOD ANALYSES[1]

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Generic relationships within Episcieae were assessed using ITS and *ndhF* sequences. Previous analyses of this tribe have focussed only on *ndhF* data and have excluded two genera, *Rhoogeton* and *Oerstedina,* which are included in this analysis. Data were analyzed using both parsimony and maximum-likelihood methods. Results from partition homogeneity tests imply that the two data sets are significantly incongruent, but when *Rhoogeton* is removed from the analysis, the data sets are not significantly different. The combined data sets reveal greater strength of relationships within the tribe with the exception of the position of *Rhoogeton.* Poorly or unresolved relationships based exclusively on *ndhF* data are more fully resolved with ITS data. These resolved clades include the monophyly of the genera *Columnea* and *Paradrymonia* and the sister-group relationship of *Nematanthus* and *Codonanthe.* A closer affinity between *Neomortonia nummularia* and *N. rosea* than has previously been seen is apparent from these data, although these two species are not monophyletic in any tree. Lastly, *Capanea* appears to be a member of Gloxinieae, although *C. grandiflora* remains within Episcieae. Evolution of fruit type, epiphytic habit, and presence of tubers is re-examined with the new data presented here.

Key words: cladistics; DNA sequencing; Episcieae; Gesneriaceae; ITS; maximum-likelihood; *ndhF.*

Cladistic analyses of DNA sequences of single genes can provide useful insights into the systematics and evolution of plants (Chase et al., 1993; Baldwin et al., 1995; Smith et al., 1997b), however, single genes may reflect gene trees and not species trees (Avise et al., 1983; Doyle, 1992; Maddison, 1995). To maximize the effectiveness of molecular data, it is best to examine DNA sequences from several independent sources (Donoghue, 1994; de Queiroz, Donoghue, and Kim, 1995; Hillis, 1995; Miyamoto and Fitch, 1995; Maddison, 1997; Soltis et al., 1998). Most recently this approach has meant examining the sequences of genes from complementary (and thus independent) portions of the genome, most notably from the chloroplast and nuclear genomes (Seelanen, Schnabel, and Wendel, 1997; Rodman et al., 1998; Baum, Small, and Wendel, 1998; Soltis et al., 1998). Although there are instances where data from different genetic sources have resulted in no immediate resolution of phylogenetic relationships (Mason-Gamer and Kellogg, 1996), many cases indicate congruence (Seelanen, Schnabel, and Wendel, 1997; Munro and Linder, 1998; Rodman et al., 1998) or at least partial congruence where discrepancies can be further examined for additional resolution or for another biological explanation (Graham et al., 1998; Lutzoni, 1998).

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The Gesneriaceae have provided several useful examples for comparing data from different sources (Smith et al., 1996). The Gesneriaceae comprise ~2500–3700 species in 120–147 genera, distributed primarily in the tropics with a few temperate species in Europe, China, and Japan (Heywood, 1978; Burtt and Wiehler, 1995). The majority of species in Gesneriaceae are herbaceous perennials, but others are annuals, shrubs, lianas, or trees. Many species (20%) are epiphytic, and Gesneriaceae rank among the top ten plant families in terms of absolute numbers of epiphytic taxa (Madison, 1977; Kress, 1986). Leaves are opposite in the majority of the subfamily, but anisophylly, leading to an apparent alternate arrangement following abscission of the smaller leaf, is common. The family is divided into two subfamilies with Gesnerioideae found almost exclusively in the neotropics and Cyrtandroideae almost exclusively in the Old World (Burtt and Wiehler, 1995; Smith et al., 1997b). The Gesnerioideae are divided further into six tribes and 60 genera (Burtt and Wiehler, 1995; Smith et al., 1997b).

The Episcieae are one of the largest tribes in Gesnerioideae and comprise 17 genera (Table 1). Morphologically they are distinct among other Gesnerioideae in their nodal anatomy (Wiehler, 1983), a character not used in a cladistic analysis of morphology (Smith, 1996). Episcieae are characterized by a three-trace trilacunar node with split lateral bundles, superior ovaries, and most members have chromosome counts of $x = 9$ [$x = 8$ in *Codonanthe* (Mart.) Hanst. and *Nematanthus* Schrader]. This combination of character states is unknown among other neotropical members of the family. Within Gesneriaceae, the tribe Episcieae has been examined more fully using the chloroplast gene *ndhF* (Smith and Carroll, 1997). These data have provided support for the monophyly of this tribe, in agreement with earlier molecular (Smith et al., 1997b) and morphological data (Smith, 1996).

TABLE 1. Species sequenced in this study with Genbank submission numbers and voucher specimens. MTJB—Montreal Botanical Garden living collection accession numbers. Letters in parentheses indicate herbarium where vouchers are deposited. Species were used in both ITS and *ndh*F analyses unless otherwise indicated.

Species	Voucher	GenBank number (*ndhF*)[c]	GenBank number (ITS)[c]
Alloplectus panamensis C. V. Morton	Smith and Carroll, 1997	GBAN-AF013685	GBAN-AF206202
Alloplectus sp.	Smith and Carroll, 1997	GBAN-AF013686	GBAN-AF206203
Alsobia dianthiflora[a] (H. E. Moore & R. G. Wilson) Wiehler	Smith and Carroll, 1997	GBAN-AF013687	NA
A. punctata (Lindl.) Wiehler	Smith and Carroll, 1997	GBAN-AF013688	GBAN-AF206204
Alsobia sp.[a]	Smith and Carroll, 1997	GBAN-AF013689	NA
Alsobia sp.[a]	Smith and Carroll, 1997	GBAN-AF013690	NA
Chrysothemis friedrichsthaliana (Hanst.) H. E. Moore	Smith and Carroll, 1997	GBAN-AF013691	GBAN-AF206205
Cobananthus calochlamys (J. D. Sm.) Wiehler	Smith and Carroll, 1997	GBAN-AF013692	GBAN-AF206206
Codonanthe elegans Wiehler	Smith et al., 1997b	GBAN-U62178	GBAN-AF206207
C. gracilis[a] (Mart.) Hanst.	Smith et al., 3721 (SRP)	GBAN-AF206196	NA
Codonanthopsis peruviana Wiehler	Smith and Carroll, 1997	GBAN-AF013693	GBAN-AF206208
Columnea ambigua (Urban) Morley	Smith and Carroll, 1997	GBAN-AF013694	GBAN-AF206209
C. byrsina[b] (Wiehler) L. P. Kvist & L. E. Skog	Smith 3408 (SRP)	NA	GBAN-AF206210
C. kalbreyeriana[b] Masters	MTJB 001408-80	NA	GBAN-AF206211
C. dissimilis[b] Morton	MTJB 002940-59	NA	GBAN-AF206212
C. ericae[b] Mansf.	Smith 3385 (SRP)	NA	GBAN-AF206213
C. minor[b] (Hook.) Hanst.	J. L. Clark s.n. (SRP)	NA	GBAN-AF206214
C. mira Morley	Smith and Carroll, 1997	GBAN-AF013695	GBAN-AF206215
C. oblongifolia Rusby	Smith and Carroll, 1997	GBAN-AF013696	GBAN-AF206216
C. pulchra[b] (Wiehler) L. Skog	MTJB 001395-57	NA	GBAN-AF206217
C. sanguinea (Pers.) Hanst.	Smith and Carroll, 1997	GBAN-AF013697	GBAN-AF206218
C. schiedeana Schlecht.	Smith et al., 1997b	GBAN-U62164	GBAN-AF206219
C. tenensis[b] (Wiehler) B. Morley	Smith 3374 (SRP)	NA	GBAN-AF206220
C. trollii[b] Mansfeld	Smith 1723 (WIS)	NA	GBAN-AF206221
Corytoplectus speciosus (Poepp.) Wiehler	Smith and Carroll, 1997	GBAN-AF013698	GBAN-AF206222
Drymonia coccinea[b] (Aubl.) Wiehler	Smith 3375 (SRP)	NA	GBAN-AF206223
D. urceolata Wiehler	Smith and Carroll, 1997	GBAN-AF013699	GBAN-AF206224
Episcia fimbriata Fritsch	Smith and Carroll, 1997	GBAN-AF013700	GBAN-AF206225
E. sphalera Leeuw.	Smith and Carroll, 1997	GBAN-AF013701	GBAN-AF206226
Nautilocalyx adenosiphon (Leeuw.) Wiehler	Smith and Carroll, 1997	GBAN-AF013702	GBAN-AF206227
Nematanthus albus Chautems, ined.	Smith et al., 3726 (SRP)	GBAN-AF206197	GBAN-AF206228
N. fritschii Hoehne	Smith et al., 3720 (SRP)	GBAN-AF206198	GBAN-AF206229
Neomortonia nummularia (Hanst.) Wiehler	Smith and Carroll, 1997	GBAN-AF013703	GBAN-AF206230
N. rosea Wiehler	Smith and Carroll, 1997	GBAN-AF013704	GBAN-AF206231
Oerstedina cerricola[a] Wiehler	Hammel 5754 (US)	GBAN-AF206199	NA
Paradrymonia aurea Wiehler	Smith and Carroll, 1997	GBAN-AF013705	GBAN-AF206232
P. densa (C. H. Wright) Wiehler	Smith and Carroll, 1997	GBAN-AF013706	GBAN-AF206233
P. fuquaiana Wiehler	Smith and Carroll, 1997	GBAN-AF013707	GBAN-AF206234
Rhoogeton viviparus Lwbg.	Kvist et al., 370 (US)	GBAN-AF206200	GBAN-AF206235
Rufodorsia major Wiehler	Smith and Carroll, 1997	GBAN-AF013708	GBAN-AF206236
Gesnerioideae: Gesnerieae			
Gesneria christii Urban	Smith et al., 1997b	GBAN-U62191	GBAN-AF206237
Rytidophyllum auriculatum Hook.	Smith et al., 1997b	GBAN-U62199	GBAN-AF206238
Gesnerioideae: Gloxinieae			
Capanea affinis Fritsch	Amaya M. & Smith 393 (COL)	GBAN-AF206201	GBAN-AF206239
C. grandiflora[a](Kunth) Decne. ex Planch.	Smith and Atkinson, 1998	GBAN-AF040145	NA
Gloxinia sylvatica (H.B.K.) Kunth	Smith et al., 1997b	GBAN-U62157	GBAN-AF206240
Koellikeria erinoides (DC.) Mansf.	Smith and Carroll, 1997	GBAN-AF013709	GBAN-AF206241
Niphaea oblonga Lindl.	Smith et al., 1997b	GBAN-U62160	GBAN-AF206242
Smithiantha cinnabarina (Linden) Kuntze	Smith and Atkinson, 1998	GBAN-AF040152	GBAN-AF206243

[a] *ndhF* only.
[b] ITS only.
[c] The prefix GBAN- has been added to each accession number to link the online version of the *American Journal of Botany* to GenBank but is not part of the actual accession number.

The Episcieae are represented by some of the more diverse morphological characters within Gesneriaceae. Whereas most Gesneriaceae are characterized by capsular fruits, many Episcieae are berry-fruited. The Episcieae also contain species with a fruit type that is intermediate between a berry and capsule. This fruit has been called a display fruit (Wiehler, 1983) and is distinguished by the dehiscence of the fleshy fruit walls to reveal a brightly colored interior pericarp that contrasts with the dull, often purple-black mass of seeds and funiculi (Wiehler, 1983).

Although previous cladistic studies within Episcieae based on *ndhF* sequences have provided important resolution regarding phylogenetic relationships and evolution of morphological features, many of the clades have remained poorly resolved or weakly supported (Smith and Carroll, 1997). For example, resolution of relation-

ships among and within several taxa that have been termed ''waste-basket'' genera were not resolved in the earlier analysis (Smith and Carroll, 1997). Among these were *Columnea* L., and *Alloplectus* Mart. In his earlier revision of Episcieae, Wiehler (1983) described several new genera and rearranged the position of many species among those already described. One group that received a major rearrangement was the *Columnea* alliance. Wiehler (1973, 1983) split this group, which initially comprised three genera (*Columnea, Alloplectus* and *Drymonia* Mart.), into eight, adding *Corytoplectus* Oerst., *Pentadenia* (Planch.) Hanst., *Trichantha* Hook., *Dalbergaria* Tuss., and *Bucinellina* Wiehler, the latter four divided from *Columnea* s. l. This split has remained controversial, and more thorough examinations of these species on the basis of morphology (Kvist and Skog, 1993; Smith, 1994; Smith and Sytsma, 1994a) and chloroplast DNA (cpDNA) restriction site analysis (Smith and Sytsma, 1994b, c) indicated that *Columnea* s.l. was best retained as a single genus and that the segregate genera of Wiehler (1983) were not monophyletic. A test of this classification system using *ndhF* sequences not only was unable to resolve the relationships of groups within *Columnea* s. l., but could not provide adequate monophyletic delimitations of *Alloplectus, Drymonia, Columnea, Corytoplectus,* and a *Neomortonia* Wiehler species included in the analysis (Smith and Carroll, 1997).

Another unexpected relationship from the cladistic analysis of *ndhF* sequences regarding Episcieae was the paraphyly of *Paradrymonia* Hanst. (Smith and Carroll, 1997). The previous study sampled only three species within this genus, one of which, *P. densa,* was included because of its unusual characteristics. *Paradrymonia densa* did not form a monophyletic group with the remaining two species of the genus and caused a paraphyletic relationship. Because of the limited sampling, it is difficult to determine whether the paraphyly of this genus was due to sampling, limited resolving power of the *ndhF* sequences, or whether *P. densa* does indeed represent an independent lineage from the remainder of the genus.

All but two genera of Episcieae have a chromosome count of $n = 9$. These are *Codonanthe* and *Nematanthus* and share a count of $n = 8$. These are the only genera in the neotropical subfamily Gesnerioideae to have a count of $n = 8$; both have centers of diversity in Brazil (Wiehler, 1983) and *Nematanthus* is endemic there (Chautems, 1984, 1988). Hybrids have been generated between the two (Wiehler, 1977), implying close phylogenetic affinity. Therefore it was surprising to find that the species of *Codonanthe* and *Nematanthus* used in the *ndhF* analysis were not only not a monophyletic group, but were not closely related (Smith and Carroll, 1997).

The present analysis seeks to resolve many of these questions that remain from the previous molecular analysis by examining a second source of molecular data. These data were gathered with the goal of (1) resolving relationships not resolved with previous data, (2) strengthening support for areas of congruence between the two data sets, and (3) providing an additional, non-cpDNA source of data in an attempt to determine whether resolved relationships from previous analyses reflect only gene trees or species trees. Additionally, it was the goal of this study to include representatives of the two genera not previously sampled in the analysis, the Guyana endemic *Rhoogeton* Leeuw. and the Central American *Oerstedina* Wiehler.

The sources of data for this analysis are the nuclear ribosomal RNA intercistronic transcribed spacer regions (ITS 1 and 2) and *ndhF*. The *ndhF* gene has been used extensively within Gesneriaceae (Smith et al., 1997a, b, 1998; Smith and Carroll, 1997; Smith and Atkinson, 1998). The use of ITS sequences for systematic studies has provided a valuable tool to resolve relationships at the species and generic levels (Baldwin et al., 1995; Möller and Cronk, 1997) where the higher levels of nucleotide substitution rates found in these regions do not create extensive homoplasy.

MATERIALS AND METHODS

The gene sequences used in this analysis were generated by thermal cycle sequencing (Innis et el., 1988) of previously amplified regions. The ITS regions and *ndhF* gene were amplified in two overlapping sections (primers 5P–2G and 3P–8P; Möller and Cronk, 1997; positions 1–1350, and 972–2044, respectively) from genomic DNA isolated from fresh, frozen, or silica-gel-dried material for most species (Smith et al., 1992). Amplification and sequencing procedures followed that of Smith et al. (1997b) used for other members of Gesneriaceae. Alignments of both ITS and *ndhF* regions were done by hand since the sequence divergence was not overly high among taxa compared and alignment by hand was easy and straightforward.

Most *ndhF* sequences were from previously published data (Smith and Carroll, 1997), but additional sequences were obtained for *Rhoogeton* and *Oerstedina,* which were not included in the previous analysis. DNA for *Rhoogeton* and *Oerstedina* was obtained from herbarium specimens (Savolainen et al., 1995) using the DNEasy Plant miniprep kits (Qiagen, Valencia, California, USA) following the manufacturer's instructions. The *ndhF* gene was amplified in two overlapping fragments using primers 172–1350R and 972–2044R. The first fragment is smaller than those reported previously since amplifying the DNA from herbarium specimens required successive amplifications using internal primers. Initial amplifications followed procedures for DNA as described elsewhere (Smith et al., 1997b) using primers 1 and 1350R for the first half and 803 and 2044R for the second half. Subsequent amplifications required the use of 172 and 972 as forward primers, although the same reverse primers resulted in successful amplifications.

This analysis focused on the relationships within Episcieae, therefore nearly all genera within the tribe and several species within some of the larger genera were sampled for both ITS and *ndhF* data sets. Two to five species each were used to represent the larger or potentially nonmonophyletic genera (*Columnea, Episcia* Mart., *Alloplectus,* and *Paradrymonia*). Two of the three species of *Neomortonia* were included in this analysis to test the monophyly of this small, but morphologically diverse genus. The species used in the analysis, voucher information, and GenBank accession numbers are included in Table 1. As some taxa have only *ndhF* or ITS data available, the taxon sampling for each analysis differed slightly. Forty-two species had sequences for ITS only, 39 for *ndhF* only, and 33 had sequences for both regions. Table 1 indicates those taxa for which only one data set was available. The data matrix contains 1.71% and 20.83% (including all indels) missing cells for *ndhF* and ITS, respectively, based on total sequence alignments.

Outgroups were selected to root the tree based on recent morphological and molecular analyses of tribal relationships within Gesneriaceae (Smith, 1996; Smith et al., 1997b). The most appropriate outgroups for Episcieae were genera representing sister tribes Gesnerieae and Gloxinieae.

Phylogenetic analysis—Phylogenetic divergence was reconstructed

using PAUP version 4.0d64 to implement parsimony (Farris, 1970; Farris, Kluge, and Eckardt, 1970; Swofford and Maddison, 1987) and maximum likelihood estimates (MLE). Parsimony has been used utilized in systematic studies and has provided numerous insights into plant evolution. With molecular data it is possible to form hypotheses on the specific rates of nucleotide substitutions and to generate trees that best fit these substitution rate models (maximum-likelihood). Both methodologies are appealing, and it would be valuable to empirically compare the methods with regard to their resulting trees. A previous analysis comparing parsimony to different models of molecular evolution in Gesneriaceae (tribes Beslerieae and Napeantheae) produced highly congruent trees (Smith, 2000), therefore it was a goal of this study to make a similar comparison on a different group of taxa.

In this analysis, trees were generated using the general heuristic option. To search for islands of equally parsimonious trees (Maddison, 1991), 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.'' All analyses were conducted with indels treated as missing data since all indels were either small (one to three base pairs) or were autapomorphic. This search strategy was used for several combinations of data sets, (1) ITS only, (2) *ndhF* only, (3) ITS and *ndhF* with all taxa from both analyses, (4) ITS and *ndhF* with only taxa common to both analyses, and (5) ITS and *ndhF* with only taxa common to both analyses and *Rhoogeton* excluded. The partition homogeneity test also was examined by partitioning the data into ITS and *ndhF* for all taxa common to both data sets and a second analysis that excluded *Rhoogeton.* The partition homogeneity test used 1000 replicates with the NNI search option and mulpars ''off.'' Attempts to use TBR and mulpars ''on'' resulted in only four replicate searches after 24 h.

Maximum likelihood estimate trees were generated for (1) ITS data, (2) *ndhF* data, (3) combined ITS and *ndhF* data with taxa in common both with, and (4) without *Rhoogeton.* Maximum likelihood searches were conducted using the heuristic search option with TBR and mulpars ''on.'' Under the MLE option, the model of Hasegawa, Kishino, and Yano (1985) was used, which allows for unequal nucleotide frequencies and differential rates for transitions and transversions. The assumed nucleotide frequencies were estimated from the data: A = 0.26661, 0.27607, 0.26620, 0.26649, C = 0.17570, 0.20148, 0.17637, 0.17579, G = 0.019341, 0.21641, 0.19365, 0.19317, and T = 0.36427, 0.30603, 0.36379, 0.36455, respectively for ITS, *ndhF,* and ITS combined with *ndhF* with and without *Rhoogeton.*

Branch support analysis was performed to examine trees that were six or fewer steps 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. Clades that persisted in strict consensus trees six steps beyond the most parsimonious trees were examined using the constraints option to search for the shortest tree that did not contain that clade. Bootstrap analysis (Felsenstein, 1985) was performed using 100 replicates with TBR and mulpars ''off.''

RESULTS

Amplification of the ITS regions resulted in fragments of ~450 bp each. Full sequences of ITS 1 and 2 were obtained from both strands using all four primers except in regions close to the primers where only a single strand was sequenced. ITS sequences were not obtained for *Capanea grandiflora, Oerstedina,* and several other species for which *ndhF* sequences had been obtained (Table 1). ITS sequences were readily aligned by hand, initiating small insertions and deletions (indels) for complete alignments. Indels were not included in any of the phylogenetic analyses due to their small size. All indels were coded as missing data. The combined ITS 1 and 2 regions resulted in a total of 306 parsimony-informative characters. Maximum parsimony analysis of these data resulted in three trees of 1822 steps each, consistency index (CI) = 0.46, and retention index (RI) = 0.54 (Fig. 1).

The strict consensus of these three trees indicates that *Columnea* is a monophyletic genus that excludes all other related genera, albeit *Drymonia, Alloplectus,* and *Neomortonia rosea* are still unresolved sister taxa and *Drymonia* is polyphyletic in this analysis. Likewise, *Alsobia punctata* is sister to *Drymonia coccinea,* a result not seen with previous analyses (Smith and Carroll, 1997). *Paradrymonia* is monophyletic and although *Neomortonia nummularia* is still close to *Episcia* as it was with *ndhF* data alone, it is sister to a more inclusive clade (Fig. 1). *Nematanthus* and *Codonanthe* are a monophyletic group as would be expected on the basis of their shared chromosome count of $n = 8$. However, the close sister-group relationship of *Codonanthe* and *Codonanthopsis* Mansf. seen with *ndhF* data (Smith and Carroll, 1997) is still present with ITS data. Lastly, *Rhoogeton,* not included in earlier analyses, is within Gloxinieae, designated as the outgroup. *Rhoogeton* itself was designated as part of the ingroup in the analysis.

To minimize computational time, the MLE analysis of the ITS data used only taxa that were common to both *ndhF* and ITS data sets (Fig. 2). The −ln likelihood was 7782.33033. In general the MLE tree is similar to the parsimony tree with the exception that both *Neomortonia* species are sequential sister groups to the *Columnea*/*Alloplectus*/*Drymonia* clade and *Corytoplectus* is sister to this more inclusive clade (Fig. 2). *Alsobia punctata* is not within the *Alloplectus*/*Drymonia* clade as it was with parsimony (Fig. 1) but is instead in a clade with *Cobananthus* Wiehler, and *Rufodorsia* Wiehler as was seen with *ndhF* data previously (Smith and Carroll, 1997).

The *ndhF* parsimony analysis differed from previous analyses of Episcieae (Smith and Carroll, 1997) with the inclusion of *Rhoogeton* and *Oerstedina* and the use of only a subset of the Gloxinieae and Gesnerieae as outgroups. The parsimony analysis resulted in 47 trees of 2196 steps each, CI = 0.46, and RI = 0.37. One of these trees is presented in Fig. 3, and the strict consensus of all trees is indicated by dashed lines. This consensus tree is much less resolved than the previous results from *ndhF* analyses (Smith and Carroll, 1997), possibly in part due to a smaller outgroup sampling and a subsequently more complete search by PAUP (only six most parsimonious trees were found in the earlier, but larger analysis with numerous outgroups and a greater taxon sampling from other tribes). This tree places both *Rhoogeton* and *Oerstedina* within Episcieae, and although the position of *Rhoogeton* is not resolved, *Oerstedina* is sister to *Rufodorsia.*

The MLE analysis of the *ndhF* data resulted in a −ln likelihood of 11 136.98922 (Fig. 4). *Rhoogeton* and *Oerstedina* are both in Episcieae as indicated by parsimony and *Oerstedina* is sister to *Rufodorsia.* The MLE analysis provides resolution for the placement of *Rhoogeton* as the sister to *Nematanthus.* This tree is otherwise similar to previously published trees of Episcieae based on *ndhF* and parsimony (Smith and Carroll, 1997) in that *Col-*

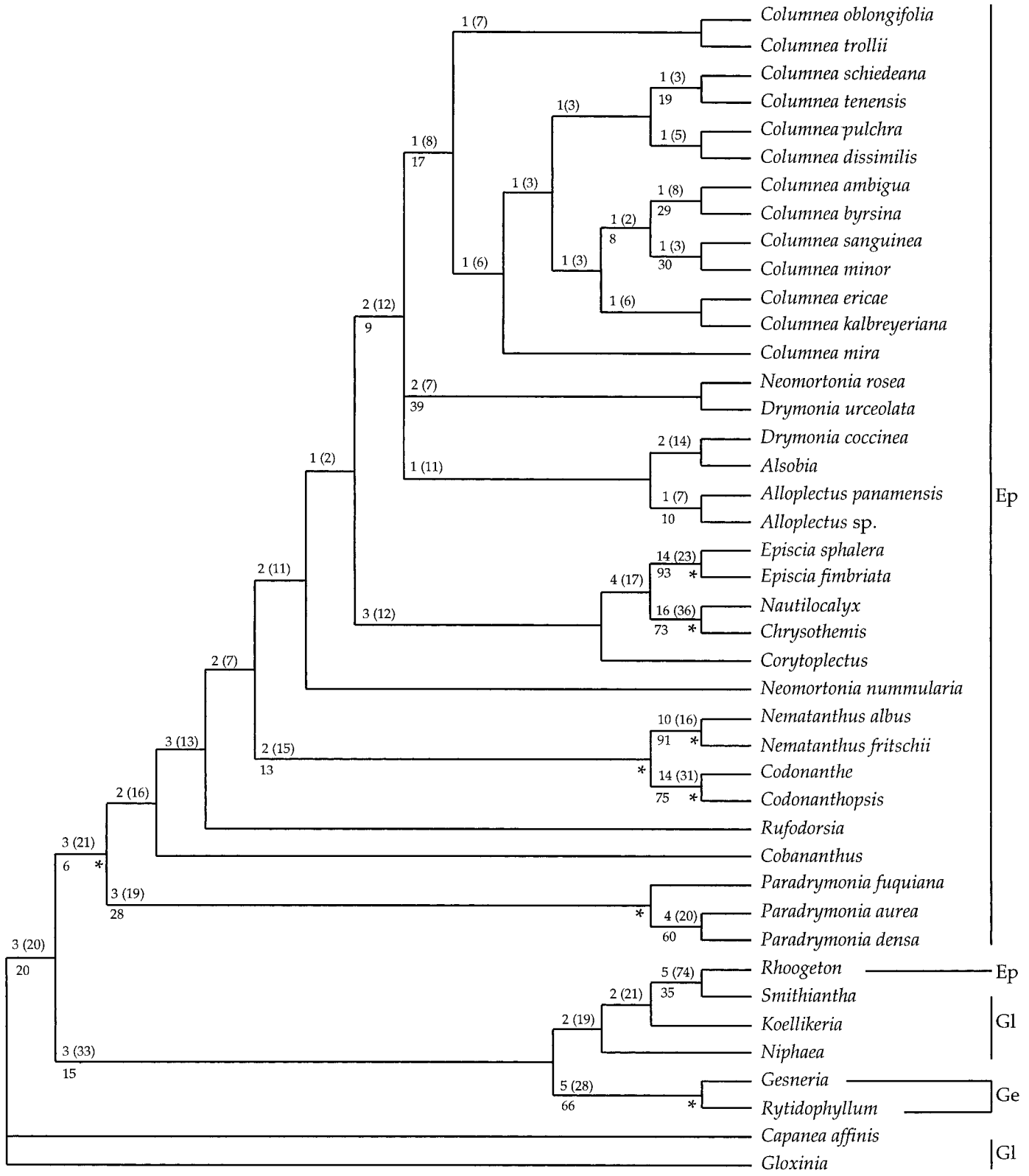

Fig. 1. Strict consensus of three trees 1822 steps each. CI = 0.46 and RI = 0.54, based on ITS sequences. These trees were rooted using Gloxinieae and Gesnerieae. Numbers above clades are decay values, and numbers in parentheses are branch lengths using the acctran option of PAUP. Numbers below clades indicate bootstrap values. Terminal branch lengths are not shown. Asterisks mark clades that are found in decay trees that include additional taxa which lack ITS sequences in a combined ITS/*ndhF* analysis (see text). Abbreviations for tribes are as follows: Ep—Episcieae, Ge—Gesnerieae, Gl—Gloxinieae.

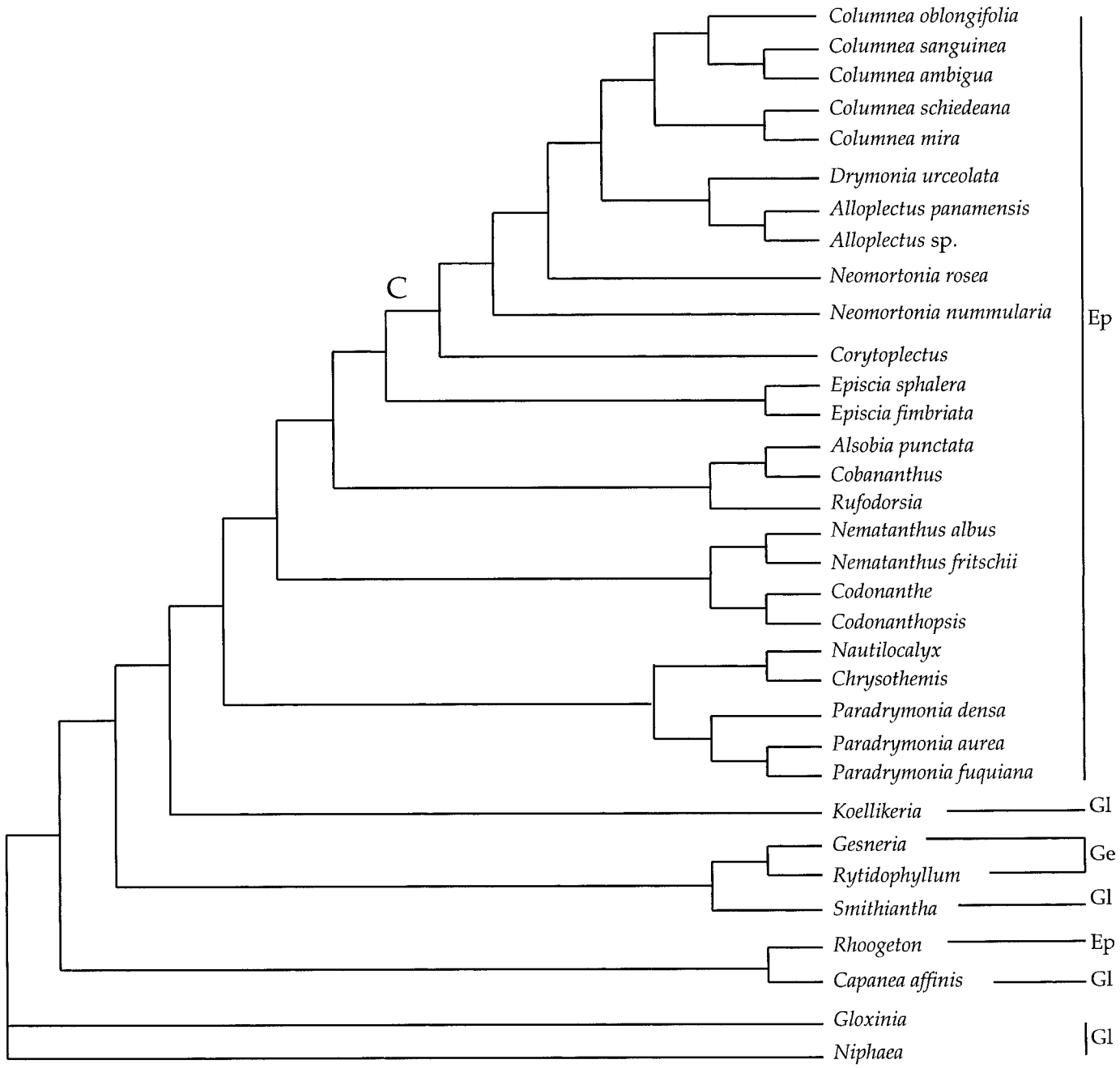

Fig. 2. Maximum likelihood estimate tree based on ITS sequences, −ln likelihood = 7782.33033, and on combined ITS and *ndhF* sequences with *Rhoogeton* excluded, -ln likelihood = 22 646.62560. To minimize computer time this analysis used only taxa common to both *ndhF* and ITS data sets. Dashed lines indicate the position of *Capanea* in the combined data analysis with *Rhoogeton* excluded. Abbreviations for tribes are as follows: Ep—Episcieae, Ge—Gesnerieae, Gl—Gloxinieae. The clade marked with a C represents the *Columnea* alliance clade (see text). The MLE tree of the combined ITS and *ndhF* data including *Rhoogeton*, -ln likelihood of 23 734.91089, is almost identical to this tree with the exception that *Rhoogeton* is within Episcieae and is sister to the *Nematanthus*/*Codonanthe* clade.

umnea is not monophyletic and contains *Alloplectus, Drymonia,* and *Neomortonia rosea.* Likewise, *Neomortonia* and *Paradrymonia* are not monophyletic and *Capanea grandiflora* is within the Episcieae.

The partition homogeneity test resulted in a *P* value of 0.001, indicating significant differences between the ITS and *ndhF* data sets. The same test resulted in a *P* value of 0.065 when *Rhoogeton* was excluded.

The combined ITS and *ndhF* data set was analyzed in three different ways since sequences for both ITS and *ndhF* were not available for all taxa. The analyses differed in that the first used all taxa with data scored as missing for the gene not sequenced, the second analysis used only taxa with both sequences, and the third used only taxa in common, but with *Rhoogeton* excluded. The first analysis resulted in 17 trees of 4151 steps each, CI

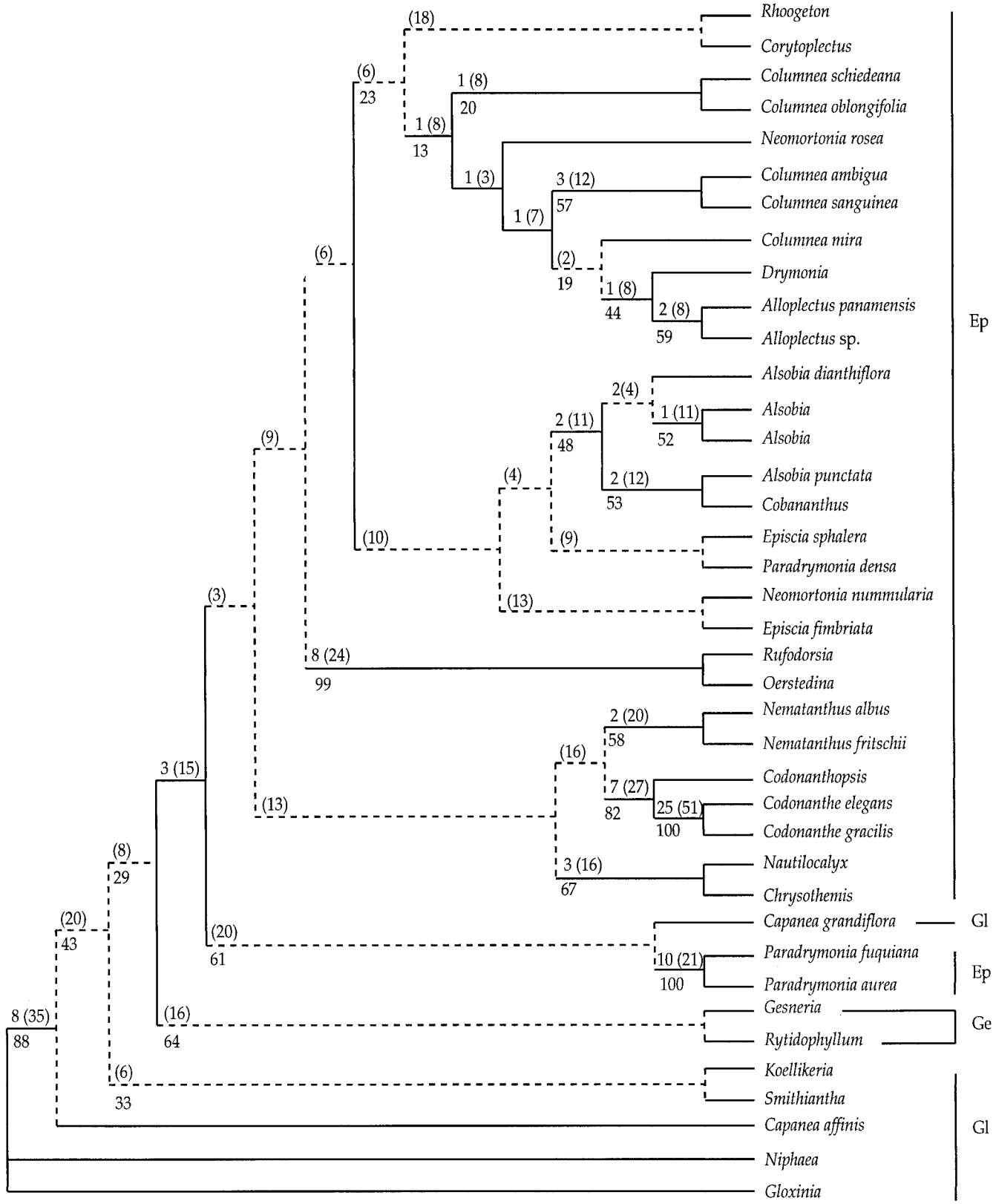

Fig. 3. One of 47 trees of 2196 steps each. CI = 0.46 and RI = 0.37, based on *ndhF* sequences. Numbers above clades are decay values, and numbers in parentheses are branch lengths using the acctran option of PAUP. Numbers below clades indicate bootstrap values. Terminal branch lengths are not shown. Branches indicated by dashed lines are not present in a strict consensus of all 47 trees. Abbreviations for tribes are as follows: Ep—Episcieae, Ge—Gesnerieae, Gl—Gloxinieae.

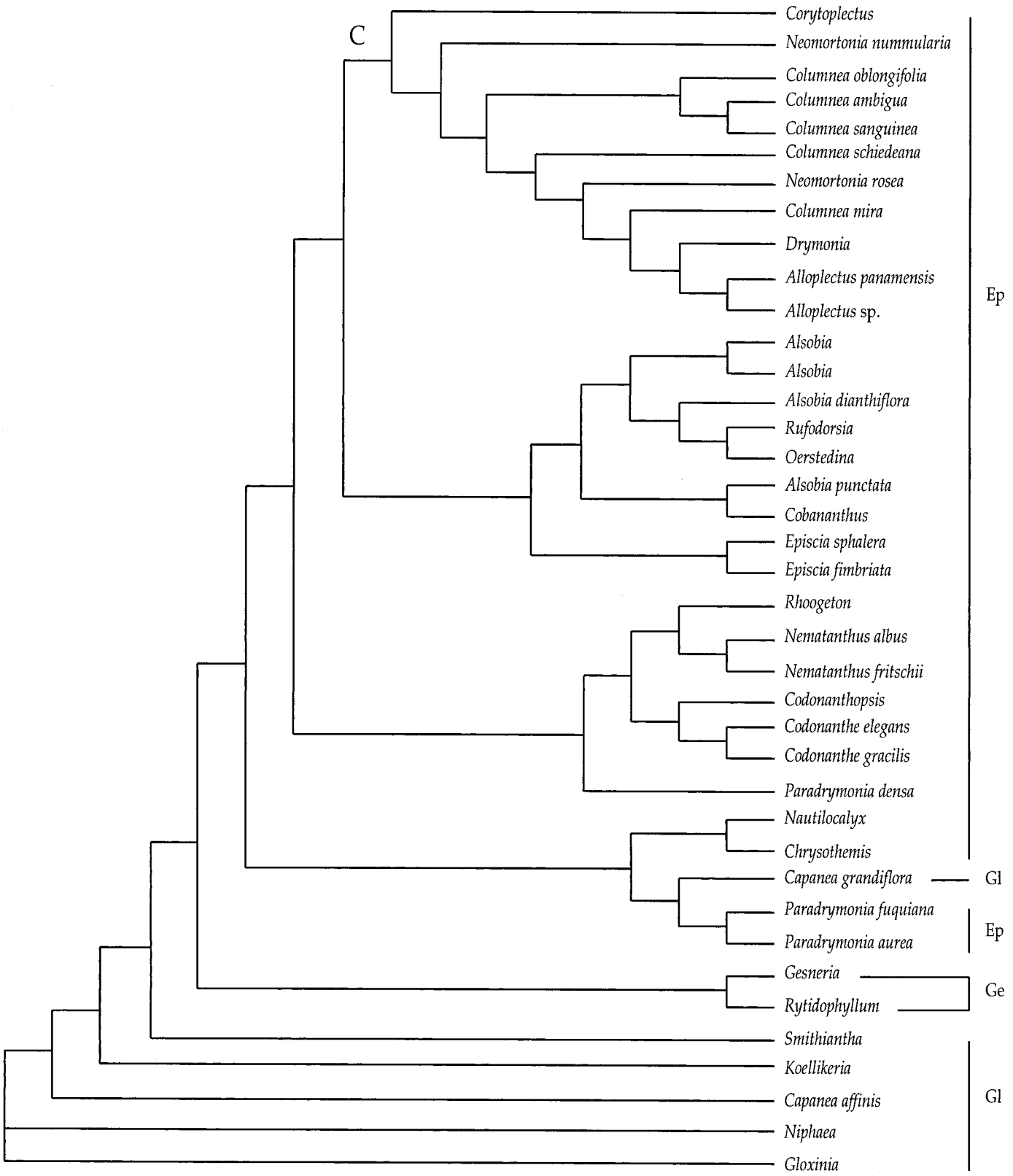

Fig. 4. Maximum likelihood estimate tree, −ln likelihood = 11 136.98922 based on *ndhF* sequences. Abbreviations for tribes are as follows: Ep—Episcieae, Ge—Gesnerieae, Gl—Gloxinieae. The clade marked with a C represents the *Columnea* alliance clade (see text).

= 0.46, and RI = 0.54. The strict consensus of this tree was less resolved than either ITS or *ndhF* data analyzed singly (tree not shown). A decay analysis resulted in only eight clades persisting in a consensus tree two steps longer than the most parsimonious tree, and these were clades that are well supported and congruent to both ITS and *ndhF* analyses (marked with asterisks in Fig. 1). A bootstrap analysis indicated only six clades with support >75%. The inclusion of *Rhoogeton* in the combined analysis resulted in a large amount of homoplasy and the combined data set tree that includes *Rhoogeton* is the least resolved tree of all data sets analyzed in this study. The second analysis, using only taxa with sequences for both ITS and *ndhF,* resulted in ten trees of 3698 steps each, CI = 0.48, and RI = 0.34. The consensus of these trees is much more resolved (Fig. 5) than the consensus tree from the first combined data analysis, and clades have better support in terms of decay and bootstrap values. The third analysis with *Rhoogeton* removed resulted in a single tree of 3467 steps, CI = 0.49, and RI = 0.51 (Fig. 5). This tree is the most well supported in terms of bootstrap, branch lengths, and decay indices. *Columnea* is delimited as a monophyletic genus separate from *Drymonia, Alloplectus,* and *Neomortonia rosea* (Fig. 5). *Paradrymonia* is monophyletic, and *Nematanthus* and *Codonanthe* are in a monophyletic clade.

The MLE tree of the combined ITS and *ndhF* data including *Rhoogeton* has a −ln likelihood of 23 734.91089. This tree is almost identical to the MLE ITS tree (Fig. 2) with the exception that *Rhoogeton* is within Episcieae and is sister to the *Nematanthus*/*Codonanthe* clade. The MLE tree of the combined data sets excluding *Rhoogeton* has a −ln likelihood of 22 646.62560 and is identical to the MLE tree based on ITS data alone (Fig. 2).

DISCUSSION

Comparison of data sets—Due to the poor level of resolution for the *ndhF* parsimony tree (Fig. 3), any comparison of this tree to the ITS parsimony tree (Fig. 1) is mostly irrelevant beyond discussion of *Rhoogeton* (below) since few other discrepant clades have any support. A comparison of the two MLE trees (Figs. 2, 4) indicates a high degree of similarity. For example, the *Columnea* alliance clade (*Columnea, Alloplectus, Drymonia, Neomortonia,* and *Corytoplectus*; marked with the letter C in Figs. 2 and 4) is similar between the two trees in terms of generic composition, although *Columnea* is monophyletic with the ITS data. In both trees, *Corytoplectus* is sister to the remainder of the clade and *Neomortonia nummularia* is sister to the remainder of the clade excluding *Corytoplectus* (Figs. 2, 4). The sister to the *Columnea* alliance clade in the *ndhF*-MLE analysis is a clade that consists of *Episcia, Alsobia* Hanst., *Cobananthus, Oerstedina,* and *Rufodorsia* (Fig. 4). The ITS tree does not include all of these genera as a single monophyletic clade sister to the *Columnea* alliance, but instead is represented by two clades (Fig. 2). The first consists of *Episcia* alone and is sister to the *Columnea* alliance clade, and the second consists of *Alsobia, Rufodorsia,* and *Cobananthus.* The latter clade is sister to the inclusive *Episcia* and *Columnea* alliance clades. The most likely explanation for this difference is the greater sampling within *Alsobia* and inclusion of *Oerstedina* in the *ndhF* analysis. Sister to these taxa in both trees is a clade composed of *Nematanthus, Codonanthe,* and *Codonanthopsis,* although *Rhoogeton* and *Paradrymonia densa* are included in this clade in the *ndhF* tree (Fig. 4). Lastly, both trees have the *Nautilocalyx* Linden ex Hanst./*Chrysothemis* Dcne./*Paradrymonia* clade as the sister to the remainder of the tribe, although the *ndhF* tree also includes *Capanea grandiflora* (see below) and lacks *Paradrymonia densa* (see below).

Despite some minor differences between the ITS and *ndhF* analyses, the overall results are similar. The main discrepancy is in the position of *Rhoogeton* as either a member of Episcieae or Gloxinieae.

Comparison of MLE and parsimony—For the most part the single tree obtained from each of the MLE analyses is one of the trees obtained from the parsimony analysis (Figs. 1–5). In a few instances the relationships are different. For example, with the ITS analysis, the two *Neomortonia* species are distant from each other with parsimony (Fig. 1), whereas they are successive sister species in the MLE analysis (Fig. 2). Similarly, *Nautilocalyx*/*Chrysothemis* is sister to *Paradrymonia* in the ITS-MLE analysis (Fig. 2), but is sister to *Episcia* with parsimony (Fig. 1). Although a cursory comparison of Figs. 3 and 4 seems to indicate numerous differences between the MLE and parsimony trees, a more careful analysis reveals that the discrepancies are only in areas of weak or no support in the parsimony tree, most being in clades that collapse in a strict consensus of all trees (Fig. 3). Therefore, the differences between these trees is largely due to the arbitrary selection of the tree represented in Fig. 3 and not any aspect of data analysis.

Combined data sets—The *ndhF* and ITS data sets provide different degrees of resolution and for the most part congruent results where both are resolved. The partition homogeneity test indicates that there is significantly different phylogenetic signal between the two data sets (P = 0.001). However, this discrepancy is eliminated when *Rhoogeton* is removed from the analysis and the partition homogeneity test instead indicates a P value of 0.065. Therefore, combining the data sets may be the best solution since the discrepancy can be traced to a single taxon. The position of *Rhoogeton* in the combined analysis must be viewed with skepticism. The uncertain position of *Rhoogeton* in the combined analysis results in a large amount of homoplasy and as a result, the combined data set tree that includes *Rhoogeton* in the analysis is the least resolved tree of all data sets analyzed in this study. This would be expected when incongruent data are combined (Bull et al., 1993; Huelsenbeck, Bull, and Cunninghan, 1996). However, when the single discrepant species is removed, the combined data tree is fully resolved, has higher consistency and retention indices, and the support for clades is higher based on bootstrap and decay values (Fig. 5). Therefore this combined data tree can be used as a basis for evolutionary analyses within Episcieae and is likely to be the best estimate of phylogenetic relationships within this tribe, although the position of *Rhoogeton* remains unresolved.

Although the basis for the incongruency between ITS

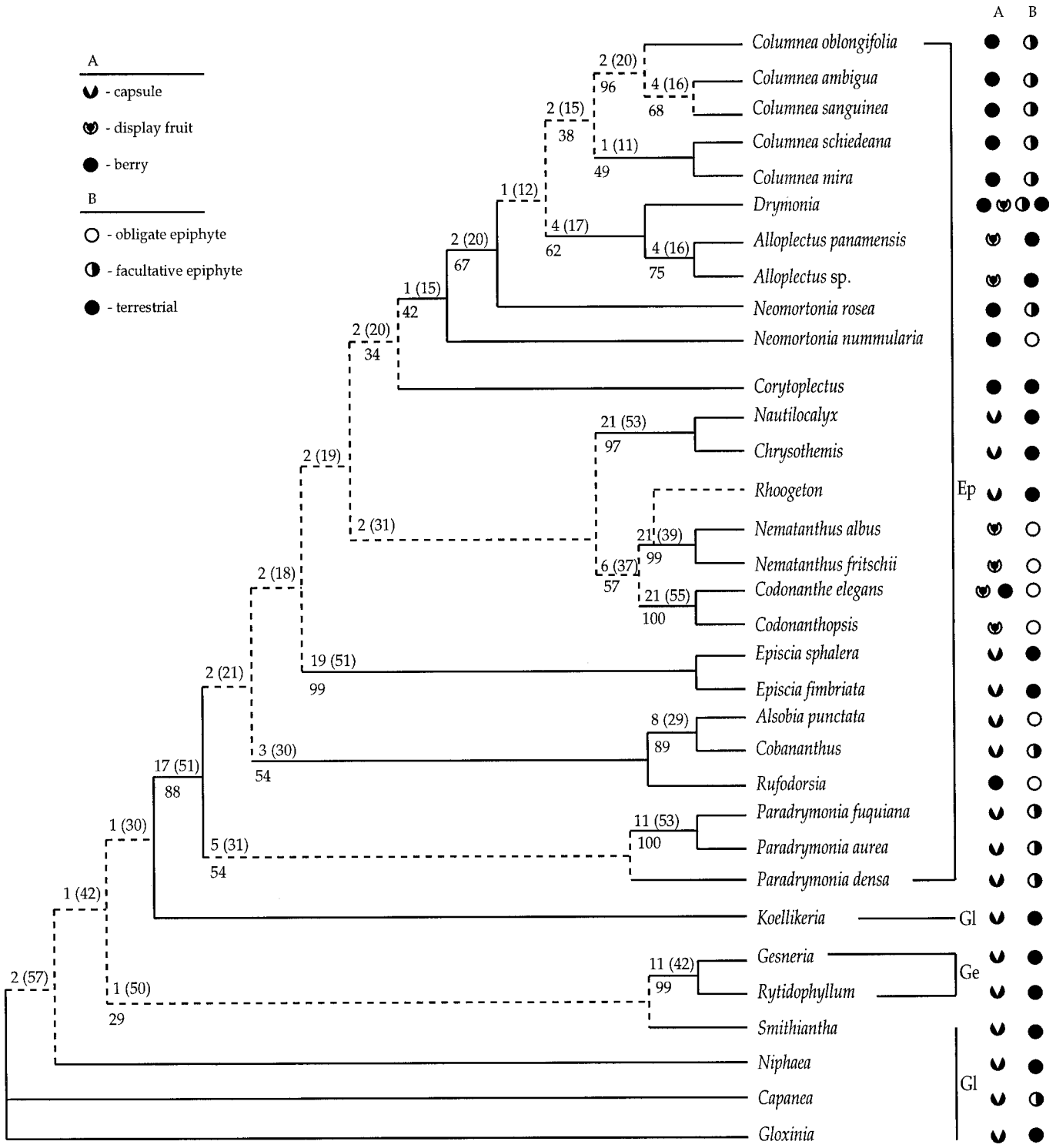

Fig. 5. Most parsimonious tree of 3467 steps, CI = 0.49, and RI = 0.51 based on the combined *ndhF* and ITS sequences with *Rhoogeton* excluded. Dashed lines represent the strict consensus of ten trees of 3698 steps each. CI = 0.48 and RI = 0.34, based on combined *ndhF* and ITS sequences with *Rhoogeton* included. *Rhoogeton* is sister to *Nematanthus* in this analysis. Numbers above clades are decay values, and numbers in parentheses are branch lengths using the acctran option of PAUP. Numbers below clades indicate bootstrap values. Terminal branch lengths are not shown. Evolution of fruit type and epiphytic habit are indicated by symbols to the right of the tree. Abbreviations for tribes are as follows: Ep—Episcieae, Ge—Gesnerieae, Gl—Gloxinieae.

and *ndhF* data for *Rhoogeton* cannot be fully resolved here, one possibility seems likely. *Rhoogeton* is isolated geographically from most other Gesneriaceae. Although other species can be found in the same general area, *Rhoogeton* is restricted to bases of water falls where the plants are continually washed in the mist and no other Gesneriaceae are present. Therefore hybridization is extremely unlikely to account for the incongruency between ITS and *ndhF* sequences in *Rhoogeton.* The species of *Rhoogeton* used in this analysis was *R. viviparus,* named because of the small plantlets that form from the edges of the leaves (Leeuwenberg, 1958). This genus has been poorly studied, therefore nothing is known regarding the relative rates by which this species reproduces sexually vs. asexually. If asexual reproduction is exclusive or predominant, it is possible that somatic mutations have accumulated in ITS sequences such that the individual sampled has diverged to the point that the sequence no longer reflects its relationships to other taxa. The *ndhF* gene may not have undergone such an extensive sequence evolution due to functional constraints. If this were true, the ITS would appear discrepant to other sequences, such as it is in this analysis.

Phylogenetic relationships within Episcieae*—The discrepant position of Rhoogeton*—The greatest discrepancy between the ITS and *ndhF* data is the position of *Rhoogeton* (Figs. 1–5) and is confirmed to be the primary source of incongruence for a significant difference between the two data sets. A higher (and not significant) *P* value of 0.065 is obtained when *Rhoogeton* is excluded from the partition homogeneity test. This genus has posed problems to previous investigators, largely due to its reduced vegetative structure and thus lack of synapomorphies to ally it to other taxa. Two species of *Rhoogeton* are found on moist, moss-covered rocks at the base of waterfalls in Guyana where they are regularly sprayed with mist. The plants are tuberous, acaulescent herbs with orange-to-red tubular corollas, a superior ovary, and an enlarged dorsal nectary gland (Leeuwenberg, 1958). A third species, *R. panamensis* Wiehler, is endemic to Panama and Costa Rica and differs by having white corollas (Wiehler, 1992, 1995). *Rhoogeton* was placed in Episcieae by Wiehler (1983) on the basis of its superior ovary and single large nectary gland. It has remained there, primarily due to a paucity of collections, inability to maintain plants in cultivation, and a lack of scientific investigation. The inclusion of *ndhF* and ITS sequences unfortunately does not provide strong evidence for its placement either within or outside of Episcieae (Figs. 1–5) since the *ndhF* data place *Rhoogeton* firmly within Episcieae and ITS data place it equally firmly outside of Episcieae and within Gloxinieae. The latter hypothesis cannot be fully examined here since additional tribes such as Sinningieae, which are also tuberous, were not sampled with ITS data. The position of *Rhoogeton* within Episcieae is maintained by *ndhF* data in studies that sample all genera of the subfamily Gesnerioideae (Smith, unpublished data), implying that it is not a limitation of sampling that causes its affinity to Episcieae.

Placement of *Rhoogeton* in either tribe has support from morphological data. The superior ovary and enlarged dorsal nectar gland are indicative of Episcieae (Wiehler, 1983). In contrast, the presence of a tuber, corolla shape, and calyx venation are all similar to *Lembocarpus* Leeuw. (Gloxinieae), an endemic genus of French Guiana and Surinam. Further studies using additional molecular regions and morphological data are necessary to resolve the position of this genus.

The Columnea alliance—In a previous analysis of Episcieae using only *ndhF* sequences, the monophyly of several genera was not resolved (Smith and Carroll, 1997). One of the larger groups of genera consisted of *Columnea, Alloplectus, Drymonia, Corytoplectus,* and *Neomortonia* (Smith and Carroll, 1997). This group of genera is herein referred to as the *Columnea* alliance. The generic delimitations of these genera and their relationships to each other are more fully resolved with ITS data (Figs. 1, 2). With the ITS data, *Columnea* is clearly delimited as a monophyletic group that does not include closely related genera such as *Alloplectus, Drymonia,* or *Neomortonia rosea* (Figs. 1, 2). However these latter taxa are not well defined, mainly due to the polyphyly of the *Drymonia* species and inclusion of *Alsobia* within this alliance based on parsimony analysis (Fig. 1). The discrepancy may be due to a limited sampling of species within *Alloplectus* and *Drymonia* (two each), both of which are moderate to large genera within Gesnerioideae. The sister relationship of *Drymonia* and *Alloplectus* is supported by *ndhF* data as well as morphological characters such as the ''display fruit'' found in both genera (Wiehler, 1983). Additional support for the monophyly of *Drymonia* comes from morphology since this genus has a unique anther arrangement within Gesneriaceae (Wiehler, 1983).

Previous phylogenetic work based on *ndhF* sequences had concluded that *Neomortonia* was not monophyletic (Smith and Carroll, 1997) and that the only morphological characters that held the species together were a laterally ''somewhat compressed orange berry'' and absence of stolons (Wiehler, 1983). Based on earlier *ndhF* sequence analysis (Smith and Carroll, 1997) *Neomortonia rosea* was proposed to be a member of the *Columnea* alliance, if not a species of *Columnea,* and *N. nummularia* was thought to be a species of *Episcia.* However, the MLE of the ITS and *ndhF* data and both parsimony and MLE analyses of the combined *ndhF* and ITS sequences indicate that the two species may be more closely related than indicated previously (Figs. 2, 4, 5). Additional sequence data may yet provide evidence that this is a monophyletic genus, and therefore its exact taxonomic status cannot be resolved at this point.

Corytoplectus was an unresolved member of the *Columnea* alliance based on *ndhF* data alone (Smith and Carroll, 1997; Fig. 3). However, with the parsimony analysis of ITS data this genus is sister to the *Episcia*/*Nautilocalyx*/*Chrysothemis* clade (Fig. 1) This position is unusual in that *Corytoplectus* has had a long taxonomic history of relationship and classification as a member of the *Columnea* alliance and no affinity to *Episcia.*

In contrast, the MLE analysis of ITS data places *Corytoplectus* as sister to the *Columnea* alliance (Fig. 2). This latter relationship is more in accordance with the traditional taxonomy for this genus as well as the *ndhF* data. The combined analysis excluding *Rhoogeton* is in agree-

ment with the MLE analysis of the ITS data (Figs. 2, 5). Additional support for the placement of *Corytoplectus* in the *Columnea* alliance is its berry fruit, which most members of the *Columnea* alliance share. Therefore this genus is best viewed as a member of the *Columnea* alliance.

Paradrymonia—Paradrymonia was polyphyletic in previous (Smith and Carroll, 1997) and present (Figs. 3, 4) analyses of *ndhF* sequences. The lack of monophyly is due to the inclusion of *P. densa,* which has long creeping stems, uncharacteristic for the remainder of the genus. The lack of monophyly for this clade based on *ndhF* sequences alone has several alternative explanations: (1) *Paradrymonia densa* should be reconsidered a separate genus, (2) *ndhF* sequences have low resolving power and are not capable of fully resolving the monophyly of *Paradrymonia,* or (3) greater taxon sampling is necessary since only three species have been sampled. The ITS data resolve this issue in that all three *Paradrymonia* species form a monophyletic group (Figs. 1, 2), implying that the low resolving power of *ndhF* sequences at this taxonomic level was the main problem in resolving the relationships of this genus and not limited taxon sampling.

The n = 8 clade—An unexpected result from previous *ndhF* analyses of Episcieae was the distant relationship of *Codonanthe* and *Nematanthus* (Smith and Carroll, 1997). Both of these genera have centers of diversity in Brazil and *Nematanthus* is endemic there (Wiehler, 1983; Chautems, 1984, 1988). In addition these are the only members of Gesnerioideae to have a chromosome count of $n = 8$, and artificial intergeneric hybrids have been generated between the two (Wiehler, 1977). The *ndhF* data, however, placed both taxa widely apart and implied that *Codonanthopsis* ($n = 9$) was the sister to *Codonanthe* (Smith and Carroll, 1997). These results imply that chromosome numbers may have general implications for phylogenetic relationships, but for more specific relationships, they were less reliable.

In the present analysis, two different species of *Nematanthus* and an additional species of *Codonanthe* have been added. All new species were collected from the wild or from cultivated material that was originally collected from the wild. With the wild-collected species included, it is apparent that the $n = 8$ species form a monophyletic group based either on *ndhF* or ITS data regardless of analysis (Figs. 1–5). *Codonanthopsis* is still sister to *Codonanthe* and is embedded within the $n = 8$ clade. Further studies of chromosomal rearrangements may clarify this, but at present, it appears that *Codonanthopsis* represents a reversal to the $n = 9$ state from within the $n = 8$ clade.

Oerstedina—This small Central American genus was not included in previous analyses of Episcieae (Smith and Carroll, 1997) as material was not available and attempts at extracting DNA from herbarium specimens had not been made at that time. Unfortunately ITS sequences were not attainable using the herbarium specimen DNA, although the *ndhF* gene was amplified and sequenced with success. *Oerstedina* was described by Wiehler (1977) as similar to *Rufodorsia* but different by having (1) larger corollas that (2) lack red coloration on the back, and (3) pointed berries. The *ndhF* data unambiguously place *Oerstedina* as sister to *Rufodorsia* in both the MLE and parsimony analyses (Figs. 3, 4) with one of the more strongly supported clades in the parsimony analysis (decay of 8, bootstrap of 99; Fig. 3). Therefore the molecular data with support from morphological characters imply a close sister-group relationship between these two genera.

Capanea—In a previous cladistic study of *ndhF* sequences within Gloxinieae and Gesnerieae (Smith and Atkinson, 1998) *Capanea grandiflora* was placed within Episcieae which was the designated outgroup for the analysis. Although this genus has traditionally been placed within Gloxinieae (Wiehler, 1983; Burtt and Wiehler, 1995), its placement within Episcieae was strongly supported based on *ndhF* sequences (Smith and Atkinson, 1998). Its position within Episcieae seemed justified in that *Capanea* was the only epiphytic member of Gloxinieae and seemed more appropriately placed amid the remaining epiphytic members of Gesnerioideae in Episcieae (Smith and Atkinson, 1998). The inclusion of *C. affinis* in both ITS and *ndhF* sequence data in this analysis does not concur with the position of *Capanea* in Episcieae and instead places *Capanea affinis* within Gloxinieae (Figs. 1–5). However, the *ndhF* data still place *C. grandiflora* within Episcieae (Figs. 3, 4). ITS sequences were not obtained for *C. grandiflora* despite numerous attempts via direct sequencing of PCR products. The unreadable ITS sequences imply that multiple copies of the ITS region may exist for this individual. One possible explanation is that a hybridization event may have carried the chloroplast genome and an ITS copy from a member of Episcieae into this individual of *Capanea*. Intergeneric hybrids are known for Gesneriaceae (Wiehler, 1968, 1977; Worley, 1979) but are not common in the wild. A cross between *Capanea* and any member of Episcieae would be unlikely due to discrepant chromosome numbers ($n = 8$ or 9 in Episcieae, $n = 13$ in *Capanea*), therefore the exact nature of the discrepant *ndhF* sequence for *Capanea grandiflora* cannot be resolved with the data available. The position of *C. grandiflora* as a species in this present analysis must be viewed with skepticism until further data can resolve the discrepancy. In contrast, the position of *C. affinis* in Gloxinieae is in accordance with the ITS and *ndhF* data presented here (Figs. 1–5) and is supported by chromosome numbers ($n = 13$ in Gloxinieae) and morphological data such as nectary structure and nodal anatomy (Wiehler, 1983).

Origin of morphological characters—A previous phylogenetic analysis of Episcieae examined the origin of several morphological characters mapped onto the cladogram produced from *ndhF* data (Smith and Carroll, 1997). With a more fully resolved and well-supported tree based on the combined data (Fig. 5), it is worthwhile to re-examine those characters on the present cladogram.

Fruit type—Three fruit types are found within Episcieae: (1) capsules, common to the majority of the subfamily, (2) berries, known only from Gesnerioideae outside the Episcieae in *Besleria* L. and the tribe Coronanthereae, and (3) the ''display fruit'' (Wiehler, 1983). The berries of *Besleria* and Coronanthereae are structur-

ally different, however, and are unlikely to be homologous to the berries of Episcieae. The display fruit is a fleshy dehiscent fruit where the interior of the pericarp is brightly colored, either red, pink, white, or yellow, and contrasts with the mass of seeds and funiculi it reveals when split open, which are usually black or blue-black (Wiehler, 1983).

All outgroup genera have capsular fruits (Fig. 5), therefore the capsular fruit is assumed to the ancestral condition for Episcieae. Other molecular-based cladistic studies of tribal relationships in Gesneriaceae have indicated that the earliest lineages in the tribe either have only capsular fruits (Smith et al., 1997b) or that the capsular fruit is ancestral to the tribe (Smith, 2000). The only exception to this is tribe Coronanthereae which has fleshy fruits that are structurally different from all other fleshy fruits in Gesnerioideae (Wiehler, 1983).

Earlier analyses indicated that berries evolved a minimum of four times and display fruits three times (Smith and Carroll, 1997). In the present analysis, both of these fruit types have one less origin each due to the monophyly of *Codonanthe, Codonanthopsis* and *Nematanthus,* where there is a single origin for both fruit types (Fig. 5). The other origins are the *Columnea* alliance and *Rufodorsia*/*Oerstedina* for berry fruits, and *Alloplectus*/*Drymonia* for display fruits (Fig. 5). As found previously (Smith and Carroll, 1997), the display fruit is always found in clades with berry fruits (Fig. 5). Poor resolution within the *ndhF* tree prevented any conclusion as to whether one fruit type preceded the other as an intermediate stage. In the present tree, the *Columnea* alliance clade provides evidence that the berry preceded the display fruit since the *Alloplectus*/*Drymonia* clade is embedded in an otherwise berry-fruited clade (Fig. 5). However, the *Nematanthus*/*Codonanthe* clade seems to imply that the display fruit preceded the berry since only *Codonanthe* has berries. However, some species of *Codonanthe* also have display fruits, and this trait may vary within individual plants of the same species (Wiehler, 1983). This implies that the berry evolved within *Codonanthe.* Further cladistic analyses within these genera will be necessary to fully resolve this, but if true, this implies that berries and display fruits have evolved following different pathways and processes of evolution within Episcieae.

Epiphytic habit—Since the Episcieae contain the majority of epiphytic species in the subfamily Gesnerioideae it is useful to examine this character on the tree. Epiphytism here is considered a complex character, being the suite of characters that permit plants to grow on trunks and branches of other plants (Smith and Carroll, 1997). As before, the most parsimonious explanation for the origin of epiphytism (including facultative epiphytes) is a single gain at the base of the tribe. Poor resolution in the *ndhF* tree did not permit an accurate estimate on the number of reversals to the terrestrial condition, which was estimated at five or six (Smith and Carroll, 1997). The present tree clearly indicates a reversal to the terrestrial condition four times with a possible fifth reversal depending on where *Rhoogeton* is placed in the tree.

The ambiguous position of *Rhoogeton* does not allow for an accurate estimate for the origin of the terrestrial condition, however its proximity to *Nautilocalyx* and *Chrysothemis* implies that it may be a part of this clade and that a single reversal to the terrestrial condition could be hypothesized for these three genera (Fig. 5).

Tubers—Additional support for the placement of *Rhoogeton* with *Nautilocalyx* and *Chrysothemis* comes from examining the origin of tubers. Tubers are known from other tribes in both Gesnerioideae and Cyrtandroideae, but within Episcieae are known only from *Drymonia, Nautilocalyx, Chrysothemis, Paradrymonia,* and *Rhoogeton.* The close proximity of *Rhoogeton* to *Nautilocalyx* and *Chrysothemis* implies that the tuber may be viewed as a synapomorphy to unite these three genera and would indicate only three origins for the tuberous condition within Episcieae.

Summary—The results from this analysis indicate that although *ndhF* data alone do not strongly resolve relationships within Episcieae, these sequences are not incongruent with ITS data and when combined provide a well-resolved and well-supported phylogenetic estimate of these genera. The only discrepancy between the data sets lies in the position of *Rhoogeton* and the inclusion of this species in both data sets results in incongruency between the two. *Rhoogeton* is well supported in the Episcieae on the basis of *ndhF* sequences but is equally well supported in the Gloxinieae with ITS data. Therefore its phylogenetic position within the subfamily Gesnerioideae remains unresolved with these data alone. MLE and parsimony were both found to produce similar results with MLE usually finding one of several most parsimonious trees. The addition of the ITS data to the analysis provides resolution of several relationships within Episcieae. The *Columnea* alliance is supported and the monophyly of *Columnea* confirmed. The two *Neomortonia* species used in this analysis are more closely related to each other with ITS sequences combined with *ndhF* than with either data sets alone (Smith and Carroll, 1997). *Paradrymonia* is most likely monophyletic, and the previous doubts on the monophyly of this genus (Smith and Carroll, 1997) are most likely the results of the limited resolving power of *ndhF* at this taxonomic level. Both *Codonanthe* and *Nematanthus* are in a monophyletic group due to the inclusion of additional species of *Nematanthus.* This grouping makes better phylogenetic sense than previous studies that had separated the genera (Smith and Carroll, 1997) since they are the only members of Gesnerioideae to have a chromosome count of $n = 8$. The present study also added the *ndhF* sequence of *Oerstedina* to the analysis that was placed as sister to *Rufodorsia,* a result in congruence with morphological data. *Capanea affinis* is best considered a member of Gloxinieae.

Morphological characters were examined on the combined data tree indicating that berry fruits evolved independently three times and display fruits evolved twice. The ancestral state for the tribe is assumed to be epiphytic with four reversals to the terrestrial condition. If *Rhoogeton* is sister to the *Nautilocalyx*/*Chrysothemis* clade, then tubers would have three origins within the tribe.

Future work on Episcieae will necessitate resolving the position of *Rhoogeton.* This will require the inclusion of additional data from both chloroplast and nuclear genes.

Additionally the inclusion of other species from this genus would enhance the analysis since other species are not known to have extensive vegetative reproduction.

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