Systematic Botany (2003), 28(2): pp. 365–375

A Preliminary Phylogeny of *Alloplectus* (Gesneriaceae): Implications for the Evolution of Flower Resupination

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Communicating Editor: James F. Smith

ABSTRACT. Monophyly of the neotropical plant genus *Alloplectus* (Gesneriaceae) was tested using maximum parsimony and maximum likelihood phylogenetic analyses of molecular sequence data from the nuclear ribosomal (nrDNA) internal transcribed spacer region (ITS). As currently circumscribed, *Alloplectus* is polyphyletic and includes taxa in three different clades. The clade that contains the type species is described as *Alloplectus* sensu stricto and is characterized by the presence of resupinate flowers. The *Alloplectus* s.s. clade is weakly supported as the sister-group to *Columnea*. A separate clade of non-resupinate ''*Alloplectus*'' species nests within a paraphyletic *Drymonia*. A third taxon, *Alloplectus cristatus*, endemic to the Lesser Antilles and northwestern South America, is also resupinate and unresolved in a basal polytomy, removed from the other species of *Alloplectus*. The fourth taxon, *Alloplectus peruvianus*, which was originally described as *Columnea peruviana*, is strongly supported as nesting within *Columnea*. Resupination of flowers is an important feature that has not been previously reported and should be recognized as a morphological synapomorphy for *Alloplectus* s.s. Within the tribe Episcieae, flower resupination is a convergent feature that is independently derived in the *Alloplectus* s.s. clade, *Alloplectus cristatus*, and a clade comprising some *Nematanthus* species.

The genus *Alloplectus* Martius has been used by Gesneriaceae systematists as a catchall group for taxa that do not fit into other genera in the tribe Episcieae. Most characters traditionally used to define *Alloplectus*, such as a fleshy bivalved dehiscent capsule, a pendent inflorescence of a reduced pair-flowered cyme, and a base chromosome number of n=9, are symplesiomorphic characters that *Alloplectus* shares with other genera such as *Drymonia* Martius and *Paradrymonia* Hanstein (Smith and Sytsma 1994a, b, c; Smith and Carroll 1997). Other characters used to define *Alloplectus* such as a tubular or ventricose corolla tube are convergent with *Nematanthus* Schrader. Thus *Alloplectus* has become a large genus that lacks well-defined morphological synapomorphies uniting all species in the genus.

Alloplectus ranges from southern Mexico to Peru with one species in the Lesser Antilles, but is most diverse in western Ecuador and Colombia. Members of *Alloplectus* are subwoody perennials that are a conspicuous component of the understory vegetation in transitional forests throughout the northern Andes, Central America, and lowlands of northwestern South America.

The Neotropical Gesneriaceae comprise one major subfamily and five tribes (Burtt and Wiehler 1995). *Alloplectus* is a member of the Episcieae, which is the most diverse tribe in the family with 21 genera and an estimated 784 species (Burtt and Wiehler 1995) or roughly 21% of all Gesneriaceae. Episcieae is also the least studied and generic concepts remain poorly defined, partly because of a simplistic use of fruit structure in delimiting genera. *Columnea* L. is the only genus in the Episcieae that has been rigorously tested and shown to be monophyletic using morphological and molecular data (Smith 1994; Smith and Sytsma 1994a, b, c). There is especially a need for more species and genus level phylogenetic analyses in Episcieae. Currently, the monophyly of most genera has not been tested using modern phylogenetic methods, very few genera are defined by morphological synapomorphies, and most probably do not represent natural groups (clades).

Alloplectus is one genus of Episcieae in need of revision, as the most recent treatment of the entire genus is over 100 years old (Hanstein 1865). Hanstein's publication focused on the Gesneriaceae at the botanical garden in Berlin and provided an overview of the family. *Alloplectus* was only a part of Hanstein's (1865) entire monograph of the Gesneriaceae known at that time, but he recognized 30 species of *Alloplectus* in his treatment. More recent estimates of the genus range from 60 (Skog 1979) to 75 (Burtt and Wiehler 1995) species.

Three phylogenetic hypotheses for the position of *Alloplectus* have been proposed in previous studies (Fig. 1), but there has not been any strong evidence supporting one hypothesis over the others. Additionally, taxon sampling in previous studies has been limited to one or two species of *Alloplectus*. Previous studies can be summarized as follows (Fig. 1):

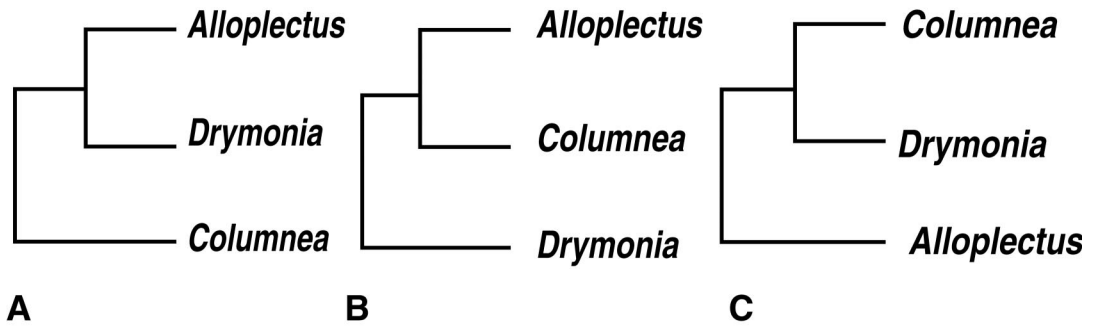

FIG. 1. Diagrammatic representation of phylogenetic hypotheses for the placement of *Alloplectus*. A. Based on molecular sequence data from *ndh*F (Smith et al. 1997; Smith and Carroll 1997; Smith 2000a) and combined analyses of the nr-ITS region (Smith 2000b). B. Based on cp DNA restriction site variation (Smith and Sytsma 1994a, b, c). C. Based on *ndhF* (Smith et al. 1997b) and combined analyses of *trnL* and ITS (Zimmer et al. 2002).

- *Drymonia* (2 spp. sampled) and *Alloplectus* (2 spp. sampled) forming a clade that is sister to C*olumnea* (13 spp. sampled) is the most commonly suggested hypothesis in recent studies. Molecular sequence data from *ndhF* (Smith 2000a; Smith and Carroll 1997; Smith et al. 1997) have supported this hypothesis (Fig. 1a) and combined analyses of the nr-ITS region and the chloroplast gene *ndhF* (Smith 2000b) have supported this hypothesis (Fig. 1a).
- *Alloplectus* (2 spp. sampled) and *Columnea* (26 spp. sampled) form a clade that is sister to *Drymonia* (2 spp. sampled) was proposed by Smith and Sytsma (1994a, b, c) using chloroplast DNA restriction site variation (Fig. 1b).
- *Columnea* (5 spp. sampled) and *Drymonia* (1 sp. sampled) form a clade that is sister to *Alloplectus* (2 spp. sampled) was proposed by Smith and Carroll (1997) using the chloroplast gene *ndhF.* This same hypothesis was also proposed by Zimmer et al. (2002) using cpDNA of the *trnL-F/trnE-T* and the nrDNA ITS region with one sample from each genus (Fig. 1c).

Recent molecular studies published for Gesneriaceae have focused on relationships at the level of genus and above. Most of these studies have relied on genes that evolve at a relatively slow rate such as the chloroplast DNA encoded gene *ndhF* (Smith and Carroll 1997; Smith et al. 1997) and the chloroplast *trnL-F* and *trnE-T* spacer region (Zimmer et al. 2002). The present molecular analysis utilized sequences of the nuclear ribosomal (nrDNA) internal transcribed spacer region (ITS), a noncoding region between 18S and 26S of the nrDNA. The ITS region is useful for species-level analyses because it is highly repeated in the plant genome, which allows for easy detection, amplification, cloning, and sequencing (Baldwin et al. 1995). The ITS region is also more variable relative to the adjacent ribosomal DNA coding regions (Baldwin et al. 1995). Although there are many copies of the gene, they undergo rapid concerted evolution, a result of unequal crossing over and gene conversion (Arnheim et al. 1980; Zimmer et al. 1980; Hillis et al. 1991) that therefore yield a clean signal in sequencing.

This study provides the first compelling evidence for the polyphyly of *Alloplectus* as currently circumscribed, and offers suggestions for a new definition of a more restricted, monophyletic group that is referred to as *Alloplectus* sensu stricto. One important discovery is that numerous species of *Alloplectus* s.s. exhibit flowers that are oriented 180° relative to all other members of the tribe, a phenomenon known as resupination.

MATERIALS AND METHODS

Taxon Sampling. Seventy-two taxa were sequenced for the nrDNA ITS region (Table 1). Most samples were from leaf material collected in the field and dried in silica gel. Other samples were from live plants growing at the Smithsonian's National Museum of Natural History Botany Research Greenhouses (Suitland, Maryland). All species were verified from live collections of flowering specimens. Currently, all taxa except for *Alloplectus cristatus* have fertile voucher specimens archived at the Smithsonian Institution's U.S. National Herbarium (US). A positive determination of *Alloplectus cristatus* was confirmed by photographs of flowers (Smithsonian Institution's Gesneriaceae Photo File).

Because monophyly of *Alloplectus* was initially considered dubious, the ingroup for this analysis comprised 70 samples including 14 of the 17 genera included in the tribe Episcieae. Large genera were sampled heavily; we used 28 samples of *Alloplectus*, 18 samples of *Columnea*, and seven samples of *Drymonia*. *Columnea* is a genus of about 200 species (Kvist and Skog 1993; Smith 1994) and *Drymonia* is a genus of about 140 species (Burtt and Wiehler 1995). Despite the apparent small sample size of seven species for *Drymonia* relative to the sample size of 28 species for *Columnea*, the latter is much more variable and is treated by some authors as five separate genera (Burtt and Wiehler 1995) instead of one genus (Kvist and Skog 1993; Smith 1994; Smith and Sytsma 1994a, b, c). Thus, a larger relative sample size was carried out to insure that this study represented the wide range of morphological variation present in *Columnea* and *Drymonia*. An attempt was also made to sample taxa from a range of geographic localities from Central America, South America, and the Caribbean.

Primary outgroups were *Sinningia incarnata* and *Sinningia cooperi*. Although these taxa are currently included in the tribe Gloxinieae by Burtt and Wiehler (1995), recent molecular phylogenetic analyses using combined data sets from cpDNA *trnL-F/trnE-T* spacer regions and the nrDNA ITS spacer region (Zimmer et al.

TABLE 1. Taxa sequenced in molecular phylogenetic study of *Alloplectus* (Gesneriaceae) with voucher, institution and GenBank accession number. US-Smithsonian Institution's U.S. National Herbarium. USBRG-Living collection from the Smithsonian Institution's Botany Research Greenhouse.

Ingroup:

Alloplectus baguensis L.E. Skog J.L. Clark 5448 (US), AF543226; *Alloplectus cristatus* (L.) Mart. USBRG-2000-191, AF543267; *Alloplectus dodsonii* Wiehler J.L. Clark 6205 (US), AF543256; *Alloplectus grandicalyx* J.L. Clark & L.E. Skog J.L. Clark 5449 (US), AF543218; *Alloplectus herthae* Mansf. J.L. Clark 4598 (US), AF543230; *Alloplectus hispidus* (Kunth) Mart. J.L. Clark 5625 (US), AF543232; *Alloplectus ichthyoderma* Hanst. J.L. Clark 5626 (US), AF543231; *Alloplectus martinianus* J.F. Sm. J.L. Clark 5793 (US), AF543228; *Alloplectus medusaeus* L.E. Skog J.L. Clark 4973 (US), AF543223; *Alloplectus panamensis* C.V. Morton J.L. Clark 5961 (US), AF543227; *Alloplectus penduliflorus* M. Freiberg J.L. Clark 6122 (US), AF543224; *Alloplectus purpureus* L.P. Kvist & L.E. Skog J.L. Clark 6100 (US), AF543222; *Alloplectus schultzei* Mansf. J.L. Clark 6039 (US), AF543219; *Alloplectus sprucei* (Kuntze) Wiehler J.L. Clark 6093 (US), AF543221; *Alloplectus tenuis* Benth. J.L. Clark 4597 (US), AF543258; *Alloplectus* aff. *tenuis* Benth. J.L. Clark 4586 (US), AF543254; *Alloplectus teuscheri* (Raymond) Wiehler J.L. Clark 5911 (US), AF543252; *Alloplectus tetragonoides* Mansf. J.L. Clark 5033 (US), AF543217; *Alloplectus weirii* (Kuntze) Wiehler J.L. Clark 5788 (US), AF543233; *Alloplectus* sp. nov. 1a J.L. Clark 4489 (US), AF543215; *Alloplectus* sp. nov. 1b J.L. Clark 4588 (US), AF543216; *Alloplectus* sp. nov. 2 J.L. Clark 6020 (US), AF543225; *Alloplectus* sp. nov. 3 J.L. Clark 5847 (US), AF543229; *Alloplectus* sp. J.L. Clark 4625 (US), AF543220

Alsobia punctata (Lindl.) Hanst. L.E. Skog 5349 (US), AY047090

Chrysothemis pulchella (Donn ex Sims) Decne. L.E. Skog 5714 (US), AY047085

Cobananthus calochlamys (Donn. Sm.) Wiehler J.L. Clark 5613 (US), AF543273

Codonanthe carnosa (Gardner) Hanst. J.L. Clark 6268 (US), AF543271

Columnea calotricha Donn. Sm. J.L. Clark 6279 (US), AF543237; *Columnea dissimilis* C.V.Morton J.L. Clark 4960 (US), AF543238; *Columnea erythrophaea* Decne. ex Houllet J.L. Clark 6273 (US), AF543246; *Columnea eubracteata* Mansf. J.L. Clark 4582 (US), AF543249; *Columnea harrisii* (Urb.) Britton ex C.V. Morton J.L. Clark 6278 (US), AF543248; *Columnea inaequilatera* Poepp. & Endl. J.L. Clark 5004 (US), AF543234; *Columnea isernii* Cuatrec. J.L. Clark 6253 (US), AF543247; *Columnea linearis* Oerst. J.L. Clark 6274 (US), AF543240; *Columnea medicinalis* (Wiehler) L.E. Skog & L.P. Kvist J.L. Clark 4482 (US), AF543235; *Columnea minor* (Hook.) Hanst. J.L. Clark 2934 (US), AF543243; *Columnea peruviana* Zahlbr. J.L. Clark 5813 (US), AF543250; *Columnea picta* H. Karst. J.L. Clark 4513 (US), AF543245; *Columnea rubriacuta* (Wiehler) L.P. Kvist & L.E. Skog J.L. Clark 4975 (US), AF543242; *Columnea rileyi* (Wiehler) J.F. Sm. J.L. Clark 6263 (US), AF543239; *Columnea schimpffii* Mansf. J.L. Clark 6280 (US), AF543236; *Columnea spathulata* Mansf. L.E. Skog 7820 (US), AY047092; *Columnea strigosa* Benth. J.L. Clark 4480 (US), AF543251; *Columnea sulfurea* Donn. Sm. J.L. Clark 6275 (US), AF543241; *Columnea zebrina* Raymond J.L. Clark 6277 (US), AF543244

Corytoplectus cutucuensis Wiehler R.W. Dunn 9405017 (US), AY047094

Drymonia conchocalyx Hanst. J.L. Clark 6276 (US), AF543261; *Drymonia crenatiloba* (Mansf.) Wiehler J.L. Clark 5462 (US), AF543259; *Drymonia hoppii* (Mansf.) Wiehler J.L. Clark 5036 (US), AF543263; *Drymonia macrophylla* (Oerst.) H.E. Moore J.L. Clark 4776 (US), AF543262; *Drymonia rhodoloma* Wiehler J.L. Clark 4843 (US), AF543260; *Drymonia serrulata* (Jacq.) Mart. L.E. Skog 7876 (US), AY047093; *Drymonia urceolata* Wiehler J.L. Clark 5225 (US), AF543265

Episcia lilacina Hanst. L.E. Skog 8132 (US), AY047091

Nautilocalyx melittifolius (L.) Wiehler L.E. Skog 7852 (US), AY047086

Nematanthus corticola Schrad. J.L. Clark 6271 (US), AF543268; *Nematanthus jolyanus* (Handro) Chautems J.L. Clark 6270 (US), AF543269; *Nematanthus strigillosus* (Mart.) H.E. Moore L.E. Skog 7751 (US), AY047089; *Nematanthus wettsteinii* (Fritsch) H.E. Moore J.L. Clark 6285 (US), AF543272; *Nematanthus* sp. nov. Chautems in ed. J.L. Clark 6266 (US), AF543270

Neomortonia nummularia (Hanst.) Wiehler J.L. Clark 6248 (US), AF543266; *Neomortonia rosea* Wiehler L.E. Skog 8113 (US), AY047096

Paradrymonia binata Wiehler USBRG 96-092, AY047087; *Paradrymonia fuquaiana* Wiehler J.L. Clark 5409 (US), AF543274; *Paradrymonia longifolia* (Poepp.) Wiehler J.L. Clark 6262 (US), AF543264

sp. nov. 1 J.L. Clark 5736 (US), AF543253; sp. nov. 2 J.L. Clark 4592 (US), AF543255; sp. nov. 3 J.L. Clark 5713 (US), AF543257

Outgroup:

Sinningia cooperi (Paxt.) Wiehler L.E. Skog 7808 (US), AY047082; *Sinningia incarnata* (Aubl.) D.L. Denham L.E. Skog 7784 (US), AY047083

2002) suggest that *Sinningia* Nees is in a clade that is sister group to a strongly supported monophyletic Episcieae (parsimony bootstrap=91%). Other phylogenetic analyses using combined data sets from *ndhF, rbcL,* and morphology (Smith 2000a) and *ndhF* (Smith 2000b) indicate that *Sinningia* is closely related, but not sister-group to Episcieae. In fact, we found that only *Sinningia* species were easily alignable for nr ITS regions. When other taxa from Gloxinieae (e.g., *Heppiella ulmifolia* (Kunth.) Hanst.) or Beslerieae (e.g., *Besleria aggregata* (Mart.) Hanst.) were included, it was difficult to align these sequences with members of Episcieae because of the increased sequence divergence relative to the more similar sequences of *Sinningia* species.

DNA Extraction, Amplification, and Sequencing. DNA was isolated using the Qiagen DNeasy™ DNA isolation kit (Qiagen, Valencia, California, USA). Templates of the nrDNA internal transcribed spacer region (ITS) were prepared using the primers ITS5HP (5′ –GGA AGG AGA AGT CGT AAC AAG G-3′; Suh et al. 1993) and ITS4 (5′ –TCC TCC GCT TAT TGA TAT GC-3′; White et al. 1990). Polymerase chain reaction (PCR) amplifications followed the procedures described by Baldwin et al. (1995) utilizing Taq DNA polymerase (Promega, Madison, Wisconsin, USA). To reduce within-strand base pairing that can result in interference with Taq polymerase activity, we found it useful to use 5% DMSO and 5% BSA in PCR reactions. The PCR products were electrophoresed using a 1.0% agarose gel in 1x TBE (pH 8.3) buffer, stained with ethidium bromide to confirm a single product, and purified using PEG 8000 (polyethylene glycol) in 2.5 M NaCl under the conditions described in Johnson and Soltis (1995). Direct cycle

sequencing of purified template DNAs followed the manufacturer's specifications, using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, California, USA). Cycle sequencing was carried out with the two initial PCR primers and the internal primers, ITS3 and ITS2 (White et al. 1990). Sequencing was performed using an Applied Biosystems Model 377 Automated DNA Sequencing System (PE Biosystems).

DNA chromatograms were proofed, edited, and contigs were assembled using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA). The sequences were truncated to include only ITS1, 5.8S, and ITS2 regions. Identification of the ends of ITS1 and ITS2 were determined by comparisons with other Gesneriaceae sequences (Zimmer et al. 2002). Sequences have been deposited in GenBank (accessions AF543215 to AF543274; Table 1).

Alignment and Phylogenetic Analyses. All sequences were initially aligned using Clustal X (Thompson et al. 1997) under the multiple alignment mode with the following parameters: gap opening = 15.00; gap extension = 6.66; delay divergent sequences = 40%; DNA transition weight = 0.50. The resulting sequences were then imported into the program SeAl version 1.0a1 (Rambaut 1996) multiple sequence editor for the final alignment. Because the sequences were not highly divergent, it was possible to make minor adjustments so that overlapping gaps were minimized. The minimization of overlapping gaps made it easier to code them as separate presence/absence characters. This approach allowed for single-site and multiple-site gaps to be treated with equal weight (Simmons and Ochoterena 2000). Tree searches were carried out with indels as missing data in the alignment, but incorporated in the final data matrix as separate characters for each gap as a presence/absence character.

The maximum parsimony (MP) analysis was performed to completion using the heuristic search options in PAUP* (Swofford 2001). The MP analysis in PAUP* was done using the following settings: 100 random addition cycles; tree bisection-reconstruction (TBR) branch swapping; steepest descent The MP analysis was limited to 100 trees of equal length for each of the 100 replicates due to the large number of equal-length trees. Other searches were explored, but did not find shorter trees using the same settings above with the following changes: 10 random addition cycles limited to 1000 trees of equal length for each of the replicates; 1000 random addition cycles limited to 100 trees of equal length for each of the replicates.

Additional tree searches were done using the parsimony ratchet analysis of WinClada (Nixon 2002) and NONA (Goloboff 1994) with NONA acting as the parsimony search engine and WinClada as the tree and data editor. Five separate tree searches were done using the parsimony ratchet analysis in WinClada using the following settings: 200 iterations per search, one tree held for each iteration, 66 characters sampled (10% of the total), amb=poly- (only uses characters that can be defined as unambiguous to support a clade), and a random constraint level of 10. Separate tree searches were performed in WinClada as suggested by Nixon (1999) since the ratchet option can sometimes get stuck on suboptimal ''islands'' and it is therefore better to perform many separate searches with fewer iterations than one larger search with more iterations. The resulting trees were swapped to completion.

Clade robustness was evaluated in PAUP* using bootstrap (Felsenstein 1985) and decay index (Bremer 1988; Donoghue et al. 1992). The bootstrap analysis used 10 random addition replicates with TBR branch swapping, saving a maximum of 10 trees for 1000 replicates. The decay index was used to examine branches that collapsed as tree length was increased by one step in conjunction with the heuristic search option in PAUP*. Each successive increase in tree length resulted in an exponential number of corresponding trees. A strict consensus tree was made for each set of trees until all branches collapsed. Additionally, a constraint tree search with a subsequent MP analysis was implemented in PAUP* to evaluate the cost of a monophyletic *Alloplectus*.

The best fitting model for the maximum likelihood analysis was chosen using Modeltest 2.1 (Posada and Crandall 1998) starting with a neighbor-joining tree. The Modeltest 2.1 analysis tests the fit of various ML models of the data set and estimates base change frequencies, proportion of variable characters, and shape of the gamma distribution, and chooses the model that best fits the data using the hierarchical likelihood ratio test (Posada and Crandall 1998). The general time reversible (GTR) model of evolution (Yang 1994) with an estimated gamma shape parameter (gamma) and estimated proportion of invariant sites (p-inv) was then used in the ML analysis (Gu et al. 1995). The assumed nucleotide frequencies were estimated from the data: A = 0.2094, C = 0.2586, G = 0.2552, T = 0.2768. The proportion of invariable sites was 0.2435, the Gamma distribution shape parameter was 0.6382, and the number of substitution types was 6. The following substitution rate matrices were estimated by Modeltest: A/C = 1.4821, A/G = 2.7394, A/T = 1.2707, C/G = 0.5256, C/T = 5.7390.

Flower resupination was mapped onto the maximum parsimony strict consensus tree in the program MacClade (Sinauer Associates, Sunderland, Massachusetts, USA) using the minimal change option.

RESULTS

DNA Sequencing and Alignment. The four ITS sequencing primers produced overlapping fragments that collectively covered the entire spacer and 5.8S rDNA regions along both strands. The aligned ITS data matrix was 649 base pairs (bp) long with 307 variable sites, of which 207 were parsimony informative. The length of the unaligned sequences varied from 614 to 636 bp. The total alignment resulted in 11 phylogenetically informative gaps that were treated as separate presence/absence characters, thus increasing the number of characters to 660 (217 parsimony informative). Although the addition of the gap characters did not change the topology, ten of the eleven gaps were parsimony informative including one significant four base indel that was present in the majority of taxa in the *Alloplectus* sensu stricto clade (Figs. 2, 3). Outgroups and basal members of the tribe contribute 83 of the 217 parsimony informative characters. When similar analyses were conducted with the three major clades + *Neomortonia nummularia*, *Cortytoplectus cutucuencis*, and *Alloplectus cristatus*, only 134 parsimony informative characters were obtained.

Maximum Parsimony Analysis. Maximum parsimony analysis of the ITS data set resulted in 1877 most parsimonious trees (length = 1019 steps, consistency index [CI] = 0.46, retention index [RI] = 0.54, rescaled consistency index [RC] = 0.30). Fig. 2 is the strict consensus of these trees. The constrained tree search for a monophyletic *Alloplectus* resulted in a tree with the length of 1045 (i.e., 36 steps longer than a most parsimonious tree).

Maximum Likelihood Analysis. The ML analysis examined 89,566 rearrangements. One tree (−ln = 6109.56859) was found (Fig. 3). The ML and MP analyses are mostly congruent except for the higher resolution produced in the ML analysis. The results differ for the basal polytomy in the strict consensus of MP trees for *N. nummularia*/*C. cutucuensis*/*A. cristatus*. The other major difference between the ML and MP analyses is the placement of the *Neomortonia rosea*/*Alloplectus hispidus*/*A. weirii* clade. The MP analysis placed this clade, which contains the type species for the genus (*Al-*

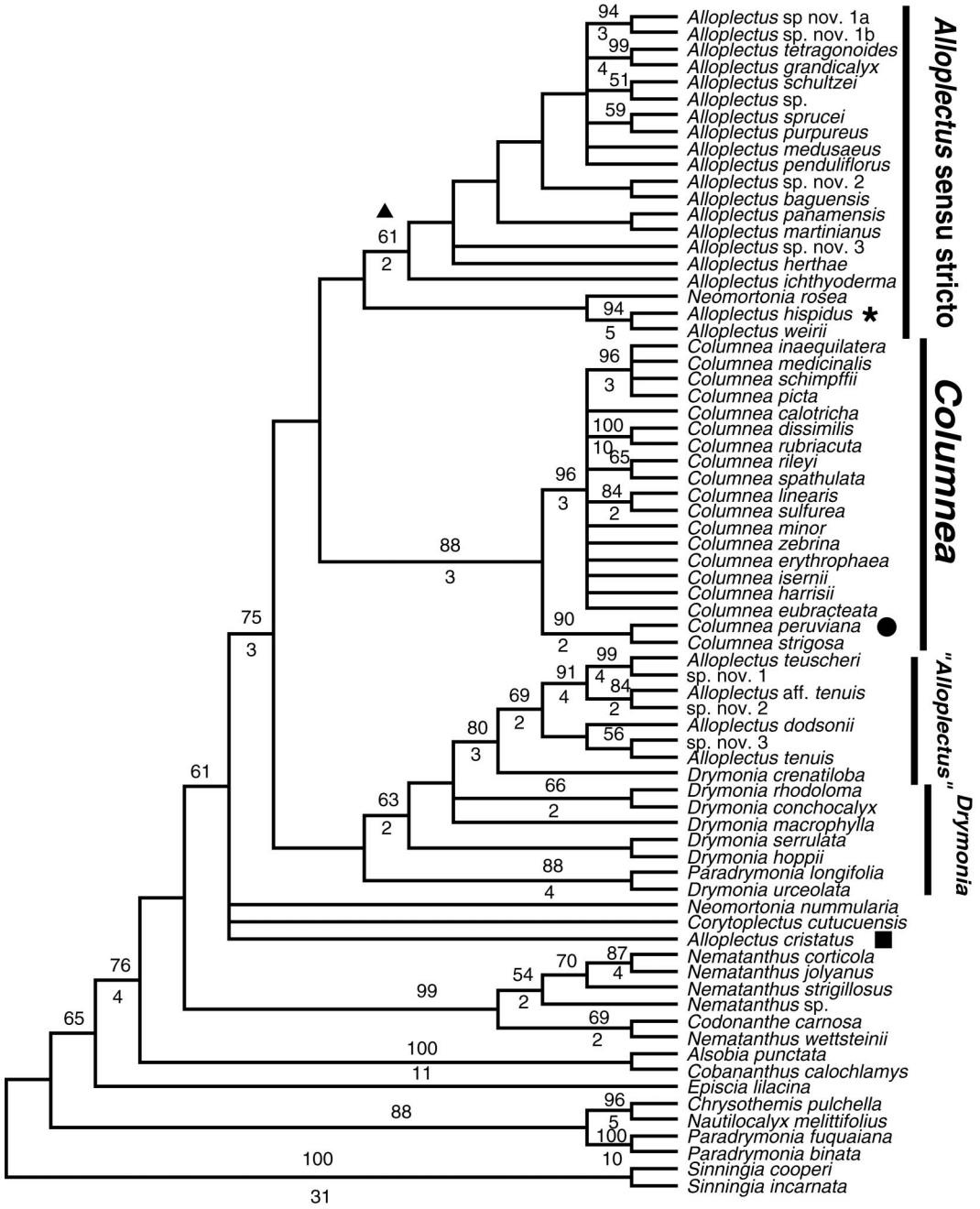

FIG. 2. Strict consensus of 1877 most parsimonious trees (length=1019 steps) from parsimony analysis of nr-ITS sequence data (CI=0.72, RI=0.59). Tree rooted with *Sinningia cooperi* and *Sinningia incarnata*. Numbers above branches are bootstrap values (>50%), those below are decay indices. * = indicates the type species for *Alloplectus*, *Alloplectus hispidus* (Kunth) Mart; ● = *Columnea peruviana* Zahlbr., previously treated as *Alloplectus peruvianus* (Zahlbr.) L.P. Kvist & L.E. Skog; ■ = *Alloplectus cristatus* (L.) Mart., non-*Alloplectus* sensu stricto species that is discussed in text; ▲= four base indel.

loplectus hispidus) with the other resupinate taxa of *Alloplectus*, whereas the ML analysis places it in a trichotomy with *Columnea*. However, bootstrap support for grouping the *N. rosea*/*A. hispidus*/*A. weirii* clade with the rest of *Alloplectus* s.s. is weak (<50% bs).

DISCUSSION

Phylogenetic Placement of* Alloplectus *sensu stricto. The phylogenetic placement of *Alloplectus* s.s. in this study is congruent with the phylogenetic

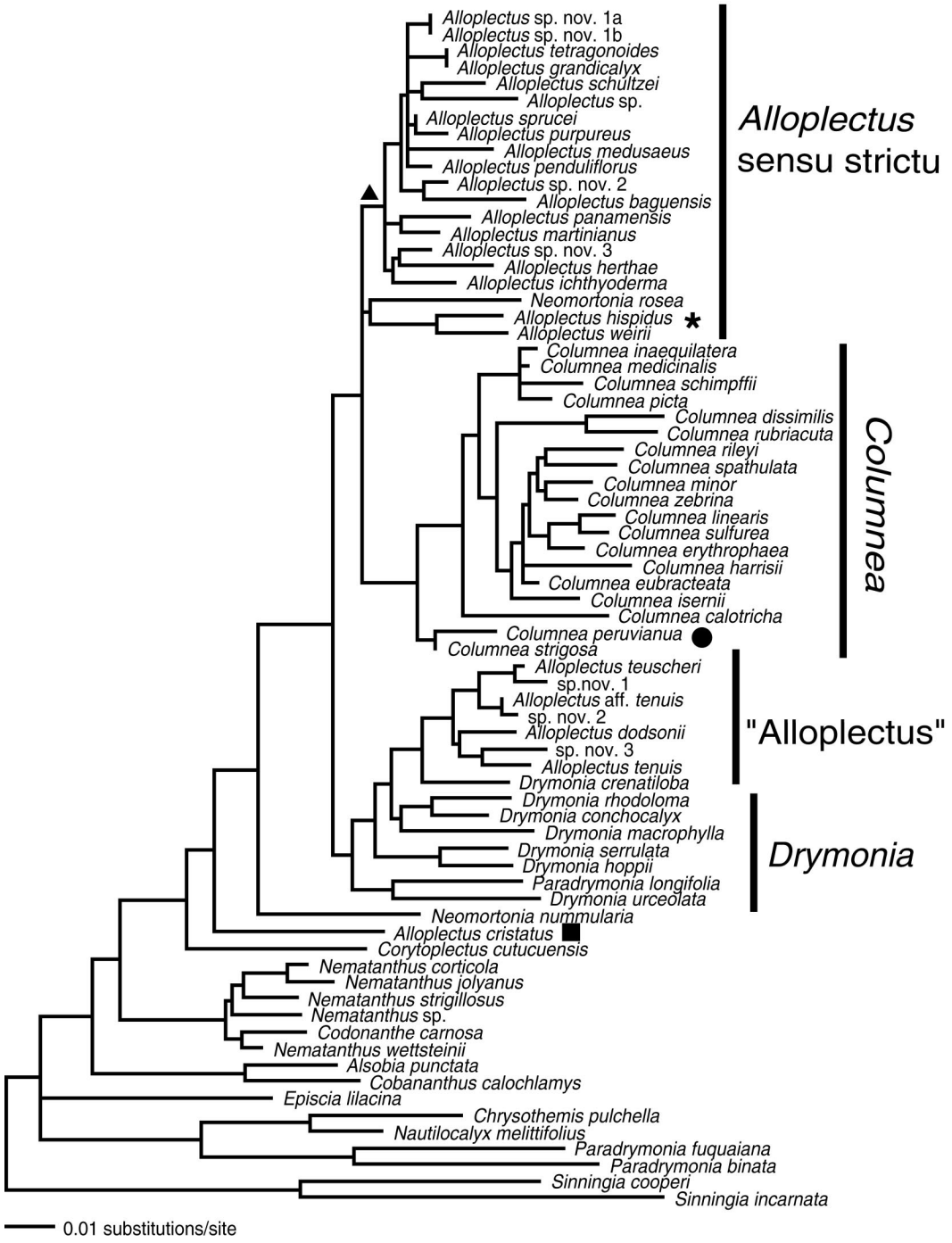

FIG. 3. Maximum likelihood estimate tree, −ln likelihood = 6109.56859 based on nr-ITS region using PAUP* (symbols same as Fig. 2).

hypothesis presented in Fig. 1B, except for the clade of *Alloplectus* species nesting with *Drymonia* (Figs. 2, 3). One important aspect of this study that helps resolve the phylogenetic placement of *Alloplectus* is an increase in taxon sampling in *Alloplectus*, *Drymonia*, and *Columnea*. No previous phylogenetic analysis using *Alloplectus* (Smith and Sytsma 1994a, b, c; Smith 1997; Smith and Carroll 1997; Smith et al. 1997; Smith 2000a; Smith 2000b; Zimmer et al. 2002) included a non-resupinate species of ''*Alloplectus*'' (i.e.,

from the non-resupinate or ''*Alloplectus*'' + *Drymonia* clade).

The placement of *Neomortonia rosea* within the *Alloplectus* s.s. clade is noteworthy because it implies an independent origin of berry fruits within a clade comprised of fleshy capsules. However, placement of *N. rosea* within *Alloplectus* s.s. is not well supported and its placement is questionable because there are no apparent morphological synapomorphies that suggest this relationship (e.g., flower orientation has not yet been observed).

The branch that places *Columnea* and *Alloplectus* together is not well supported in this analysis (bs < 50%), but the branch that subtends the three major clades, *Alloplectus* s.s., *Columnea*, and ''*Alloplectus*'' + *Drymonia*, is moderately supported (bs=75%, decay value=3). Therefore, it is possible that the relationship between these three clades could change with more data, but the overall clade will likely remain monophyletic. It should also be noted that the relatively low bootstrap values (Fig. 2) are due to few characters on the branches rather than characters that are in conflict with each other.

A new circumscription is suggested for *Alloplectus* (i.e., *Alloplectus* sensu stricto), based on the clade that contains the type species. A monographic revision of *Alloplectus* (J.L. Clark, in preparation) will include only those species that nest in this clade, while others will need to be transferred either to *Drymonia* or described as a new genus. The results presented here are preliminary and therefore nomenclatural changes will be made after more taxa are evaluated. For example, we have not been able to evaluate some of the non-Andean taxa currently in *Alloplectus*. A manuscript is in preparation that will accommodate all of the nomenclatural changes necessary after more data are evaluated (e.g., morphology) and additional taxa are added to the analyses.

***Implications for Circumscription of Non-resupinate* "Alloplectus".** The placement of the non-resupinate ''*Alloplectus*'' species in *Drymonia* is well supported (bs=80%, decay value=2). One taxon that nests within this group is *D. crenatiloba*, a poorly known species from the eastern slopes of the Andes. The generic placement of this taxon in *Drymonia* is uncertain. Although this species was originally described by Mansfeld (1938) as belonging to *Alloplectus*, Wiehler (1981) transferred it to *Drymonia* because of the presence of poricidal anther dehiscence. However, based on flower dissections from the collections of *D. crenatiloba* at the Smithsonian Institution's National Museum of Natural History, all were observed to have longitudinal dehiscence, but this should be verified with living material. Poricidal anthers in *Drymonia* are sometimes difficult to detect because anthers can dehisce initially by pores and then develop into longitudinal slits. Therefore, it is possible that the observed longitudinal slits are from a late stage in anthesis that has already matured past a younger ''pore'' stage.

Drymonia is one of the largest genera of Gesneriaceae in the Neotropics with an estimated 140 species (Burtt and Wiehler 1995). The putative morphological synapomorphy that distinguishes *Drymonia* from most other Gesneriaceae is the poricidal anther dehiscence first described by Moore (1955) and further elaborated by Wiehler (1983). The presence of poricidal anthers in most species of *Drymonia* could be a synapomorphy that is reversed in the non-resupinate ''*Alloplectus*,'' Alternatively, these taxa could be placed as sister groups when more *Drymonia* species are included in the analysis. The strong support for a monophyletic ''*Alloplectus*'' + *Drymonia crenatiloba* to the exclusion of all other *Drymonia* species is well supported. More *Drymonia* species will need to be included in future analyses before any taxonomic decisions of generic placement are made for the non-resupinate ''*Alloplectus*'' species. Based on the results presented here, a monophyletic *Drymonia* would include the non-resupinate ''*Alloplectus*'' species. Because *Drymonia* is morphologically variable and not well studied, we recommend that generic transfers wait until more species of *Drymonia* and *Alloplectus* can be included.

Circumscription and Phylogenetic Placement of* Columnea peruviana *(formerly* Alloplectus peruvianus*). Based on the results from this study, *Alloplectus peruvianus* should be transferred to *Columnea*, as *Columnea peruviana* Zahlbr. (i.e., the basionym for this taxon). There is strong support for the inclusion of this taxon in *Columnea* (bs=88%, decay value=2). Furthermore, this is congruent with Smith and Sytsma's (1994b) study based on cpDNA restriction site variation, where *Columnea peruviana* (''*Alloplectus peruvianus*'') was placed as a basal member of the genus *Columnea*. This species was transferred from *Columnea* to *Alloplectus* by Kvist and Skog (1993) because of the presence of a capsular fruit. The inclusion of this taxon in *Columnea* makes it the only known species in *Columnea* with a capsular fruit instead of a berry. In Episcieae, fleshy capsules are plesiomorphic and berries have arisen multiple times within the tribe (e.g., *Neomortonia*, *Columnea*, *Corytoplectus*, and *Codonanthe*). It is equivocal whether berries evolved once in *Columnea* with a reversal in *Columnea peruviana*. Alternatively, the fleshy capsular fruit in *C. peruviana* could be basal in the clade and berries could have independent origins in *Columnea strigosa* and the remaining species of *Columnea*. The placement of *Columnea peruviana* is not surprising because it shares many features with other members of the clade such as a five lobed nectary and a scandent habit. The presence of a five lobed nectary is not known to exist in any species of *Alloplectus* sensu stricto or the *Drymonia* + ''*Alloplectus*'' clade. Five

lobed nectaries are found in some genera of the tribe Gloxinieae (e.g., *Kohleria*, *Gloxinia*, *Pearcea*) and the *Sinningia* clade (e.g., *Sinningia*, *Paliavana*, *Vanhouttea*), but are rare in the tribe Episcieae. Most genera in the tribe Episcieae have a bilobed gland or two large dorsal connate glands. The only other genus of Episcieae besides *Columnea* with a five lobed nectary gland is *Corytoplectus* Oersted.

One feature that has been observed in basal members of *Columnea* (e.g., *C. trollii* and *C. oblongifolia* not included in this analysis) is an indehiscent fleshy fruit (berry) that when forcefully squeezed would dehisce like a capsule (Jim Smith, personal communication). Although this feature is present in basal members of *Columnea*, it has not yet been studied in *C. strigosa*. This is potentially significant because this feature could be an intermediate condition between a capsule and a berry.

***Circumscription and Phylogenetic Placement of* Alloplectus cristatus.** At present, the phylogenetic placement of *Alloplectus cristatus* is not well resolved. Based on fieldwork by the first author in Dominica and Martinique the flowers were documented as resupinate. There is strong support to exclude *Alloplectus cristatus* from the four main clades identified in this study (i.e., *Nematanthus* + *Codonanthe*; *Alloplectus* sensu stricto; *Columnea*; and ''*Alloplectus*'' + *Drymonia*). The phylogenetic placement of *Alloplectus cristatus* will most likely be resolved in future molecular and morphological analyses that expand sampling to include all genera in the tribe Episcieae (i.e., *Rhoogeton* Leeuwenberg, *Rufodorsia* Wiehler, *Oerstedina* Wiehler, and *Codonanthopsis* Mansfeld). Additional sampling of non-Andean species currently included in large genera such as *Paradrymonia*, *Drymonia*, and *Alloplectus* may help in resolving the position of this enigmatic taxon that is only known from the Caribbean and non-Andean regions of NW South America. Until more data can be included, we suggest that the generic placement of this taxon remain in *Alloplectus*.

Despite limited taxon sampling in other large genera, a few other significant phylogenetic conclusions from this study should be emphasized.

1) *Paradrymonia* is polyphyletic. Smith (2000b) also showed *Paradrymonia* to be polyphyletic based on a sample size of three species. Our study used the dubiously placed taxon *Paradrymonia longifolia*, which was initially thought to be a *Drymonia* because of the more typical campanulate flower and isophyllous opposite leaf arrangement. Most species of *Paradrymonia* have a strongly anisophyllous leaf structure and urceolate or hypocyrtoid corollas.

2) *Neomortonia* Wiehler is polyphyletic. Smith (2000b), Smith and Carroll (1997), and Zimmer et al. (2002) also showed that *Neomortonia* is polyphyletic. *Neomortonia* is a genus with 2–3 species that range from Southern Mexico to Ecuador (Wiehler 1995). The flowers of the two species used in this study (*N. nummularia* and *N. rosea*) do not look similar; *N. nummularia* is bright red and hypocyrtoid (pouched) whereas *N. rosea* is yellow and campanulate. The main characters that were used to differentiate *Neomortonia* from other genera are the presence of a berry fruit instead of the more common capsular fruit and the lack of ability to hybridize *Neomortonia* species with other members in the tribe Episcieae (Wiehler 1975). Based on the results of this study as well as others (e.g., Smith 2000b, Smith and Carroll 1997, Zimmer et al. 2002) the berry fruit of *Neomortonia* is convergent and the 2–3 species currently in this genus should be transferred to other genera.

3) *Alsobia* Hanstein and *Cobananthus* Wiehler are in a clade that is well supported and separate from the genus *Episcia* Martius. The results from this study are congruent with Smith (2000b), which recognized *Alsobia* and *Episcia* as separate genera. It is difficult to compare this relationship with Zimmer et al. (2002) because *Cobananthus* was not included in their analyses. Although *Cobananthus* was not included in Zimmer et al. (2002) *Alsobia* and *Episcia* were weakly supported as monophyletic and treated as one genus (i.e., *Episcia*).

Flower Resupination. The discovery of resupinate flowers in the *Alloplectus* sensu stricto clade provides a significant morphological synapomorphy that helps define a monophyletic *Alloplectus*. Flower resupination has never been evaluated phylogenetically for Gesneriaceae and until this study was not known to exist in *Alloplectus*. To our knowledge, the first mention of resupination in the literature for Gesneriaceae was by Chautems (1988) in his revision of *Nematanthus* and an earlier treatment of the same genus by Moore (1973). Resupination was also documented in *Sinningia sellovii* (Mart.) Wiehler and *Sinningia sulcata* (Rusby) Wiehler (Boggan 1991). Although not mentioned in the literature, resupinate flowers can be seen in photos of live plants of *Capanea grandiflora* Decne. ex Planch (front cover of The Gloxinian 36, no. 5, 1986) and *Capanea affinis* Fritsch (Smithsonian Institution's Gesneriaceae Photo File). Flower orientation is difficult to evaluate from photos and even more difficult to evaluate from herbarium specimens because there is no obvious twist in the pedicels. Most flowers of *Alloplectus* are held nearly horizontal, making the flower orientation relatively easy to evaluate, but a few are pendent therefore making it difficult to differentiate the ventral and dorsal surfaces of the corolla tube. For this reason, fieldwork was essential for accurately determining flower orientation in all the plants used in this study.

The result of floral resupination is usually a 180° rotation that gives the flower an upside down orientation. The mechanism of resupination can be attributed to the twisting or turning of the ovary, pedicel, or

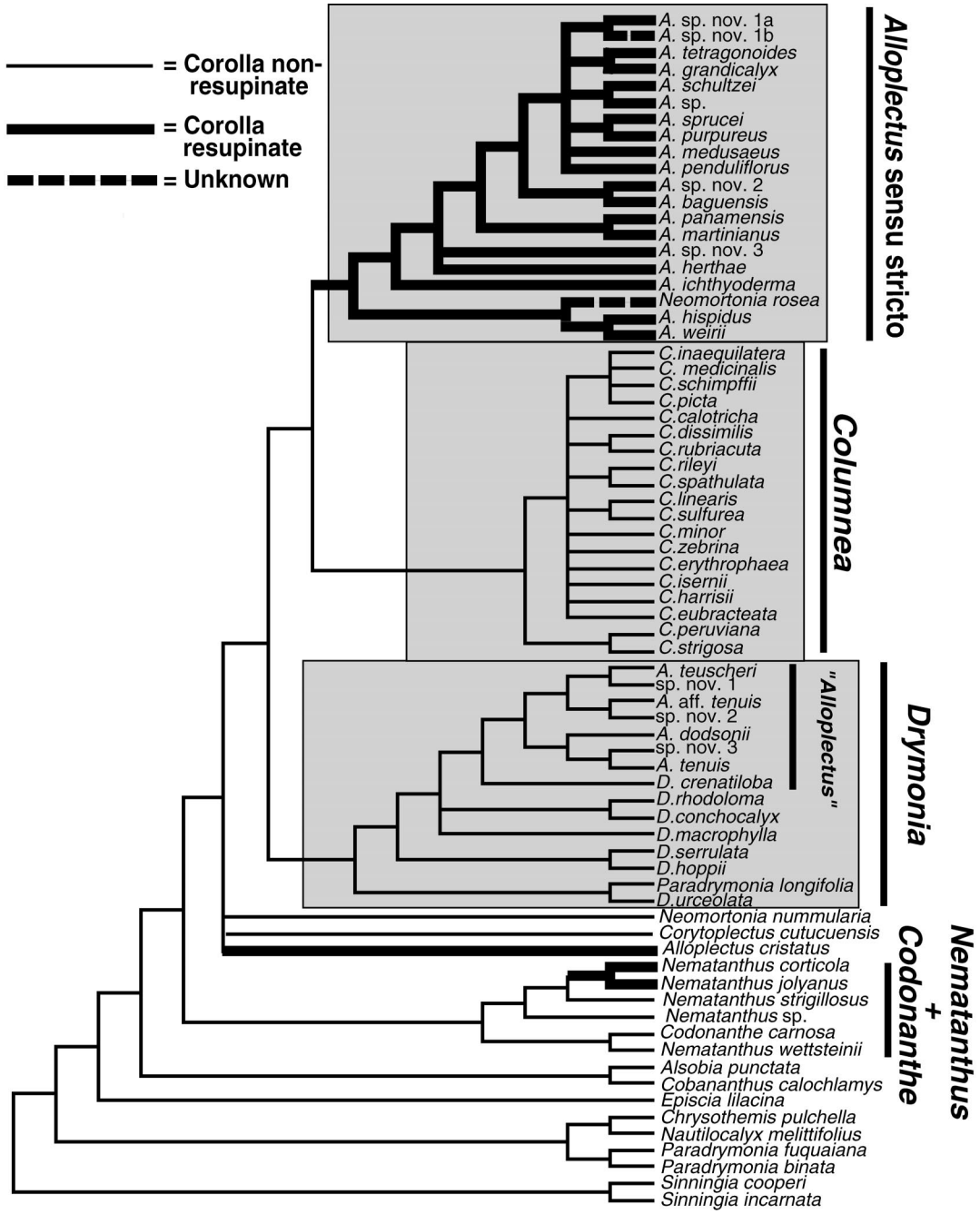

FIG. 4. Flower resupination mapped onto the maximum parsimony strict consensus tree.

both (Nyman et al. 1984). The mechanism of resupination in *Alloplectus* is unknown and currently being studied in members representing all clades presented in this study. The presence of a twisted pedicel is not obvious on herbarium sheets. Paraffin embedding, sectioning, and light microscopy techniques are being used to determine the mechanism of rotation.

Flower resupination has been well documented and studied in Orchidaceae (e.g., Ames 1838; Darwin 1892; van der Pijl and Dodson 1966; Dressler 1981; Nyman et al. 1984; Ernst and Arditti 1994), but remains relatively unknown in other groups. Other groups that are phylogenetically defined by flower resupination are the Diclipterinae in Acanthaceae (McDade et al. 2000), the subgenus *Stenochlamys* in Musaceae (Andersson 1985), and the family Lobeliaceae (Lammers 1992).

Resupinate flowers occur in three lineages in the tribe Episcieae: the *Alloplectus* sensu stricto clade, *Alloplectus cristatus*, and some members of the genus *Nematanthus* (Fig. 4). This character has remained unknown because it is difficult to verify flower orientation from herbarium specimens and most of these plants are difficult to grow. The presence or absence of resupinate flowers was verified in the field or greenhouse for all species except where noted in Fig. 4 (marked as uncertain). Thus, the flower orientation of some species such as *Neomortonia rosea* will remain undetermined until they can be observed in the wild.

All outgroup taxa and most members of the tribe Episcieae are non-resupinate. Therefore, it is assumed that non-resupinate flowers are ancestral for Episcieae. The results from this analysis suggest that resupinate flowers evolved a minimum of three times as independent gains in the *Alloplectus* sensu stricto clade, *Alloplectus cristatus*, and some *Nematanthus*. The alternative and less parsimonious explanation is that resupinate flowers are symplesiomorphic and that non-resupinate flowers resulted from losses in at least seven lineages.

The *Nematanthus* + *Codonanthe* clade is well supported in this study (bs=99%) as well as other molecular phylogenetic analyses (Smith 2000b; Zimmer et al. 2002). The *Nematanthus* + *Codonanthe* group is mostly centered in southeastern Brazil and is characterized by a base chromosome count of n=8. All other Episcieae groups have a chromosome count of n=9 (Smith 2000b; Zimmer et al. 2002). Chautems (1988) and Moore (1973) used the presence of resupinate flowers as a convenient character for differentiating groups of taxa. In his monographic revision of the genus, Chautems further divided the resupinate flowered *Nematanthus* species into those that had pendent resupinate flowers with long pedicels (2–20 cm) and those that have non-pendent resupinate flowers. In Chautems' treatment of *Nematanthus* there are nine species that are non-pendent resupinate, seven species that are pendent and resupinate, and ten species that are not resupinate. Species from each of Chautems' categories were used in this analysis. Taxon sampling in *Nematanthus* was limited to three non-resupinate (*N. wettsteinii*, *N. strigillosus*, and *Nematanthus* sp.) and two resupinate species (*N. corticola* and *N. jolyanus*). Results from this study suggest that presence of resupinate flowers in *Nematanthus* is a synapomorphy for a clade within *Nematanthus* to the exclusion of other non-resupinate *Nematanthus* species and *Codonanthe*.

Further sampling of non-Andean members of the tribe Episcieae will most likely help resolve the placement of *Alloplectus cristatus*. The three most important taxa that are not included in this analysis, but will hopefully be observed and collected in the near future are *Alloplectus tigrinus* (H. Karst.) Hanst., *Alloplectus spectabilis* Wiehler ex L.E. Skog & Steyerm., and *Alloplectus savannarum* C.V. Morton. The presence of resupination in all three of these species is currently unknown and difficult to evaluate from the limited herbarium collections available.

In conclusion, future analyses will expand taxon sampling with more emphasis on non-Andean species, which will help resolve the placement of *Alloplectus cristatus*. The phylogeny presented here is preliminary and future work will include morphology and additional molecular data from a chloroplast region. The species previously placed in *Alloplectus*, *Columnea peruviana*, is well supported as belonging in the genus *Columnea* despite its lacking berries. The recognition that *Alloplectus* is not monophyletic will facilitate further detailed analyses of other genera in the tribe Episcieae and will provide a basis for a monographic revision that the first author is currently in the process of completing.

ACKNOWLEDGEMENTS. Support for this project was provided by the American Gloxinia and Gesneriad Society, the National Science Foundation (DEB 0206512), the Explorers Club Washington Group, the George Washington University Chapter of the Society of Sigma Xi, the Cosmos Club Foundation, the American Society of Plant Taxonomists, and the José Cuatrecasas Botanical Fund of the Department of Systematic Biology—Botany, U.S. National Museum of Natural History. Initial fieldwork by the first author was conducted while he was serving as a U.S. Peace Corps Volunteer in Ecuador. We would also like to thank Molly Nepokroeff, Gery Allan, Eric Roalson, and Ken Karol from the Smithsonian Institution's Laboratories of Analytical Biology for advice on molecular technique and analyses. We are also grateful to Robert and Diane Stewart for use of plant collections from their greenhouse in Stow, MA. Finally, we are especially grateful to Ingi Agnarsson, Paula DePriest, Eric Roalson, Mark Fishbein, Patrick Herendeen, and an anonymous reviewer for helpful comments on the manuscript and figures.

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