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# A Phylogenetic Analysis within Tribes Gloxinieae and Gesnerieae (Gesnerioideae: Gesneriaceae)

JAMES F. SMITH,[1,5] SCOTTIE B. DRAPER,[1] LENA C. HILEMAN,[2,4] and DAVID A. BAUM[3]

[1]Department of Biology, Boise State University, 1910 University Drive, Boise, Idaho 83725;
[2]Harvard University Herbaria, 22 Divinity Avenue, Cambridge, Massachusetts 02138;
[3]Department of Botany, University of Wisconsin-Madison, 430 Lincoln Drive, Madison, Wisconsin 53706
[4]current address: Yale University, Osborn Memorial Labs, P. O. Box 8104, New Haven, Connecticut 06520-8104
[5]Author for Correspondence (jfsmith@boisestate.edu)

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**ABSTRACT.** The evolution of floral symmetry in Asteridae has been of recent interest. Three genera with full or partial radial floral symmetry are found within tribes Gesnerieae and Gloxinieae of Gesneriaceae where the remaining taxa have bilaterally symmetrical flowers. These tribes have been shown to each be monophyletic and to be sister to each other, but a full analysis within each tribe has not demonstrated strongly supported sister group relationships among genera, especially those with radially symmetrical flowers. To assess sister group relationships more accurately, a phylogenetic analysis using *GCYC* (a Gesneriaceae homolog of *CYCLOIDEA*) sequences as well as four regions from the chloroplast genome (*ndhF, trnL* intron, *trnF-trnL* spacer, *rpl20-rps12* spacer), ITS, and two paralogues of the nuclear encoded chloroplast expressed glutamine synthetase was conducted. The analyses for the most part resolved sister group relationships between radial and bilateral taxa with strong support as well as provided support for several clades within Gloxinieae and indicated that *Phinaea* is not a monophyletic genus.

Floral symmetry is a trait of systematic and ecological importance in Asteridae (Cronk and Möller 1997; Donoghue et al. 1998) and aspects of symmetry in Asteridae are thought to be dependent on a small set of floral symmetry genes (Luo et al. 1996; Almeida et al. 1997; Reeves and Olmstead 1998). By studying mutant plants of *Antirrhinum majus L.* (snapdragon) that lack the wild type floral bilateral symmetry, Luo et al. (1996, 1999) identified two loci that are essential for the development of bilaterally symmetrical flowers. These are *CYCLOIDEA* (*CYC*) and *DICHOTOMA* (*DICH*). The developmental aspects of these flower symmetry genes in *Antirrhinum* have stimulated interest in the evolution of plant groups with both bilateral and radial floral symmetry (Coen and Nugent 1994; Coen et al. 1995; Coen 1996; Cronk and Möller 1997; Endress 1997; Running 1997; Donoghue et al. 1998; Baum 1998; Möller et al. 1999; Citerne et al. 2000; Theißen 2000; Cronk 2002; Cubas 2002; Gillies et al. 2002; Hileman and Baum 2003; Hileman et al. 2003; Smith et al. 2004).

One family that manifests shifts in floral symmetry is Gesneriaceae (Cronk and Möller 1997). Gesneriaceae and nearly all close relatives of the family (placed in Lamiales sensu APG 1998) have bilaterally symmetrical flowers. Phylogenetic analyses (Smith 1996, 2000a; Smith et al. 1997) indicate that the ancestral condition for Gesneriaceae is bilateral symmetry and that radially symmetrical flowers have been derived several times from within otherwise bilaterally symmetrical flowered clades (Cronk and Möller 1997; Möller et al. 1999; Smith 2000a). Three genera with part or full radial floral symmetry are found within tribes Gloxinieae and Gesnerieae (*Bellonia*, *Phinaea*, and *Niphaea*). *Bellonia* exhibits full radial symmetry with five to sometimes six petals and corresponding five to sometimes six stamens. *Phinaea* and *Niphaea* have radially to slightly bilaterally symmetrical corollas, but have the typical stamen abortion seen in bilaterally symmetrical flowers and have only four stamens. Although these genera have been proposed to be closely related (Zhaoran and Skog 1990), molecular phylogenetic analyses have not indicated a close relationship among them. However, these recent phylogenetic analyses have not sampled fully from the generic diversity in the two tribes, particularly genera that are likely to be sister to radially symmetrical flowered genera (Smith and Atkinson 1998; Smith et al. 2004).

Gloxinieae and Gesnerieae have each been shown to be monophyletic and sister to one another (Smith et al. 1997; Zimmer et al. 2002; Smith et al. 2004). However, hypotheses of relationships within each of the tribes have not been strongly supported, likely the result of minimal taxon sampling (Smith et al. 1997; Zimmer et al. 2002; Smith et al. 2004) and/or insufficient data (Smith and Atkinson 1998). To overcome these two problems, we conducted phylogenetic analyses of nearly all genera currently placed in Gloxinieae and Gesnerieae. Our analyses were based on eight different DNA regions from the chloroplast and nuclear genomes. Only two genera were not included, *Gloxinia* sensu stricto from Gloxinieae (Zimmer et al. 2002) and *Pheidonocarpa*, a monotypic genus from Gesnerieae.

## MATERIALS AND METHODS

Taxa sampled for this investigation, their voucher numbers, and GenBank accession numbers for sequences are listed in Table 1. Previous phylogenetic analyses of tribes Gloxinieae and Gesner-

TABLE 1. Species sequenced in this study with Genbank submission numbers and voucher specimens. SI = Smithsonian Institution living collection. Letters in parentheses indicate herbarium acronyms where vouchers are deposited. Data are presented in the following sequence: species name, voucher, GenBank accession numbers for *ndhF,* ITS, *ncpGS1, ncpGS2, trnL* intron, *trnL-trnF* spacer, G*CYC, rpl20-rps12.*

*Achimenes cettoana* H. E. Moore, SI 94-235, AY623155, AY623371, AY623176, AY623220, AY623265, AY623295, AY623134, AY623325. *Alloplectus panamensis* C. V. Morton, *Skog et al. 7641* (US), AF013685, AF272160/AF272161, AY623215, AY623260, AY623283, AY623305, AY623933, AY623366. *Anodiscus xanthophyllus* (Poepp.) Mansf., *Dunn s.n.* (SRP), AF040143, AY623381, AY623189, AY623234, AY623275, AY623305, AY623142, AY623339.

*Bellonia spinosa* Sw., *Evans s.n.* (SRP), AF040144, AY372334/AY372351, AY623202, AY623247, AY364278, AY364300, AY363924, AY623353.

*Capanea affinis* Fritsch, *M. Amaya M. & Smith 393* (COL), AY623163, AY623383, AY623193, AY623237, AY623277, AY623307, AY623144, AY623343. *Capanea* sp., *J. L. Clark 2446* (US), AY623158, AY623375, AY623182, AY623227, AY623269, AY623299, AY623137, AY623332. *Columnea byrsina* (Wiehler) Kvist & L. Skog, *Smith 3408* (SRP), AY364308, AF272176/AF272177, AY623214, AY623259, AY623282, AY364304, AY363931, AY623365. *Cremersia platula* Feuillet & L. Skog, *J. J. de Granville s.n.* (CAY), AY623173, AY623398, AY623212, AY623257, AY623292, AY6233222, AY623152, AY623363.

*Diastema racemiferum* Benth., *Skog 7574* (US), U62156, AY372324/AY372342, AY623192, AY623236, AY364268, AY364290, AY363916, AY623342.

*Eucodonia andrieuxii* (DC.) Wiehler, *Smith 4037* (SRP), AF040146, AY623374, AY623179, AY623223, AY623268, AY623298, AY623136, AY623328.

*Gesneria christii* Urban, SI 94-507, U62191, AF272230/AF272231, AY623191, NA, AY364280, AY364302, AY363923, AY623341. *G. pedicellaris* Alain, *Smith 3950* (SRP), U62192, AY623393, AY623206, AY623251, AY623287, AY623317, AY623151, AY623357. *G. ventricosa* Sw. *J. L. Clark 6545* (US), AY623167, AY623390, AY623203, AY623248, AY623284, AY623314, AY623226, AY623354. *G. viridiflora* Kuntze, *L. A. Hahn 440* (SRP), AY623168, AY623391, AY623204, AY623249, AY623285, AY623315, AY626227, AY623355. *G.* sp. *L. A. Hahn 407* (SRP), AY623169, AY623392, AY623205, AY623250, AY623186, AY623316, AY623150, AY623356.

*Gloxinia gymnostoma* Griseb., *J. L. Clark 6804* (US), AY623164, AY623384, AY623194, AY623239, AY623278, AY623308, AY623145, AY623345. *G. purpurascens* (Rusby) Wiehler, *J. L. Clark 6805* (US), AY623165, AY623385, AY623195, AY623240, AY623279, AY623309, AY623146, AY623346. *G. sylvatica* (H.B.K.) Kunth, *Dunn 9012051* (SRP), U62157, AY372329/AY372347, NA, AY623238, AY623273, AY364295, AY363917, AY623344.

*Goyazia rupicola* Taubert, *Smith et al. 3722* (SRP), AF257485, AY372329/AY372347, AY623199, AY623244, AY364273, AY364295, AY363922, AY623350.

*Heppiella verticillata* (Cav.) Cuatr., *Smith 3427* (SRP), AF040147, AY623389, AY623201, AY623246, AY623283, AY623313, AY623921, AY623352.

*Koellikeria erinoides* (DC.) Mansf., *Smith 3953* (SRP), AF013709, AY623388, AY623200, AY623245, AY623282, AY623312, AY623149, AY623351. *Kohleria hirsuta* Regel, SI 94-472, AY623159, AY623376, AY623183, AY623228, AY623270, AY623300, AY623138, AY623333. *K. spicata* (Kunth) Oerst., *Skog 7701* (US), U62181, AY372327/AY372345, AY623184, AY623229, AY364271, AY364293, AY363919, AY623334.

*Lembocarpus amoenus* Leuw., *Smith 4125* (SRP), AY623174, AY623399, AY623213, AY623258, AY623293, AY623323, AY623153, AY623364.

*Monopyle macrocarpa* Benth., no voucher, U62197, AY623387, AY623198, AY623243, AY623281, AY623311, AY623148, AY623349. *M. maxonii* Morton, SI 94-231, AY623162, AY623382, AY623190, AY623235, AY623276, AY623306, AY623143, AY623340. *Moussonia elegans* Decne., *Smith 4031* (SRP), AY623156, AY623372, AY623177, AY623221, AY623266, AY623296, AY623228, AY623326. *M. fruticosa* (Brandegee) Wiehler, *Smith 4390* (SRP), AY623157, AY623373, AY623178, AY623222, AY623267, AY623297, AY623135, AY623327.

*Nematanthus albus* Chautems, *Smith et al. 3726* (SRP), AF206197, AF272212/AF2722143, AY623216, AY623261, AY346281, AY364303, AY363928, AY623367. *N. fritschii* Hoehne, *Smith et al. 3720* (SRP), AY623175, AY623400, AY623217, AY623262, AY623294, AY623324, AY623154, AY623368. *Niphaea oblonga* Lindl., *Skog 7564* (US), U62160, AY373326/AY372344, NA, AY623225, AY364270, AY364292, 363918, AY623330.

*Pearcea hypocyrtiflora* Regel, *Smith 3943* (SRP), AF040150, AY623378, AY623186, AY623231, AY623272, AY623302, AY363913, AY623336. *P. intermedia* L. P. Kvist & L. E. Skog, *Smith 3425* (SRP), AF040149, AY623377, AY623185, AY623230, AY623271, AY623301, AY623139, AY623335. *Phinaea albiflora* Rusby, SI 94-503, AF040151, AY372322, AY623197, AY623242, AY364266, AY364288, AY363914, AY623348. *P. divaricata* (Poepp.) Wiehler, *Smith 4397* (SRP), AY623160, AY623379, AY623187, AY623232, AY623273, AY623303, AY623140, AY623337. *P. ecuadorana* Wiehler, *Smith 4033* (SRP), AY623161, AY623380, AY623188, AY623233, AY623274, AY623304, AY623141, AY623338. *P. multiflora* Morton, *Smith 4398* (SRP), AY623166, AY623386, AY623196, AY623241, AY623280, AY623310, AY623147, AY623347.

*Rhytidophyllum auriculatum* Hook., SI 94-524, U62199, AF272232/AF272233, AY623208, AY623252, AY364279, AY364301, AY363927, AY623358. *R. leucomallon* Hanst., *Smith 3949* (SRP), AY623170, AY623395, AY623209, AY623254, AY623289, AY623319, AY363925, AY623360. *R. tomentosum* (L.) Mart., SI 77-235, U62200, AY623394, AY623208, AY623253, AY623288, AY623318, AY363926, AY623359. *R.* sp. 1, *L. A. Hahn 417* (SRP), AY623171, AY623396, AY623210, AY623255, AY623290, AY623320, AY623229, AY623361. *R.* sp. 2, *L. A. Hahn 435* (SRP), AY623172, AY623397, AY623211, AY623256, AY623291, AY623321, AY626230, AY623362.

*Sinningia richii* Clayb., SI 94-554, U62186, AY372338/AY372355, AY623219, AY623264, AY623285, AY364307, AY363935, AY623370. *S. speciosa* Hiern., *Smith 4512* (SRP), AY364309, AY372337/AY372354, AY623218, 263, AY364284, AY364306, AY363942, AY623369. *Smithiantha cinnabarina* (Linden) Kuntze, SI 94-484, AF040152, AY372323/AY372341, AY623180, AY623224, AY364267, AY364289, AY363915, AY623329. *Solenophora obliqua* D.L. Denham & D.N. Gibson, *Breedlove 71542* (CAS), U62202, AY373328/AY372346, AY623181, AY623226, AY364272, AY364294, AY363921, AY623331.

ieae have shown the sister group to be Episcieae, Sinningieae, or a clade that unites these two tribes (Smith et al. 1997; Smith 2000a; Zimmer et al. 2002; Smith et al. 2004). Therefore representatives of both tribes were included with two species of Sinningieae designated the outgroup in all analyses.

DNA was extracted either with a modified CTAB procedure (Smith et al. 1992) or using QIAGEN DNeasy extraction kits following the manufacturer's instructions. Eight DNA regions were selected for examination. These were the low copy number nuclear genes, G*CYC* (Möller et al. 1999; Citerne et al. 2000; Smith et al. 2004), and the chloroplast expressed nuclear encoded glutamine synthetase (*ncpGS*; Emshwiller and Doyle 1999, Perret et al. 2003); the chloroplast genes *ndhF* (Smith et al. 1997; Smith and Carroll 1997; Smith and Atkinson 1998; Smith 2000a, b, c, 2001), the *trnL* intron, the *trnL-trnF* spacer (Möller et al. 1999; Eldenäs and Linder 2000; Sweeney and Price 2000; Yen and Olmstead 2000; Brouat et al. 2001; Manktelow et al. 2001; Salatino et al. 2001), and the *rpl20-rps12* spacer (Hamilton 1999), and the nuclear ribosomal internal transcribed spacers, ITS (Suh et al. 1993; Baum et al. 1994; Baldwin et al. 1995; Soltis and Kuzoff 1995; Yuan et al. 1996; Möller and Cronk 1997; Smith et al. 1998; Smith 2000b; both ITS 1 and 2 have been combined in this study, but exclude the 5.8S ribosomal gene sequences). Details of amplification procedures for *ndhF* and ITS of Gesneriaceae can be found in Smith et al. (1997), and Smith (2000b), respectively. Amplification of the *trnL* intron and *trnL-trnF* spacer used the primers of Taberlet et al. (1991) and amplification procedures identical to those for *ndhF* (Smith et al. 1997). The primers and amplification protocols of Möller et al. (1999) were used to amplify G*CYC,* with an additional primer described in Smith et al. (2004). Procedures for the amplification of *ncpGS* followed the amplification protocols of Emshwiller and Doyle (1999) using their primers GS687f and GS856r. All bands amplified for the low copy nuclear genes were cloned and four to eight clones were sequenced for all species to verify that multiple alleles were not present for the species surveyed. Additional clones of ITS were made for some species where direct sequencing did not produce a clear sequence.

Sequences were obtained from both strands of all gene regions examined in this study via PCR amplification and sequencing with a Li-Cor LongreadIR 4200 automated sequencer. For most genes, except *ncpGS,* and for most samples of G*CYC* and ITS, direct sequencing provided clear sequences with few ambiguities. The pGEM-T vector system kit (Promega) was used to clone *ncpGS,* some G*CYC,* and some ITS regions where ambiguities were present in the direct sequencing. Two to eight clones each were sequenced when G*CYC* and ITS needed to be cloned. Including indels, 6.5% of the cells in the data matrix were missing. Sequences were aligned manually with only a minimum of ambiguously aligned sequences, particularly for ITS or the *rpl*20-*rps*12 region. The ambiguously aligned regions were excluded from the phylogenetic analyses. The datasets have been deposited in TreeBASE (study accession number: S1091; matrix accession number M1866).

The data were analyzed using PAUP* 4.0b10 (Swofford 2000) utilizing both maximum parsimony (MP) and maximum likelihood (ML). Indels were either excluded from the analysis such that the range of bases involved were deactived, or rescored as multi-state characters depending on the alignment of the terminus of each gap (Simmons and Ochoterena 2000). Additionally, for some gaps that had a common 3′ and 5′ terminus, the sequence composition (in taxa without the gap) differed. These were rescored as multi-state characters with each unique sequence assigned a unique state. For G*CYC,* which lacks introns, indels were rescored as multi-state characters using the amino acid translation as a template and scoring characters as groups of three base pairs corresponding to codons (Smith et al. 2004). For example, an indel of nine base pairs would be scored as three different characters, corresponding to the amino acid translation for the taxa where sequence was present. For the MP analyses, each data set was analyzed independently using the search option of Olmstead and Palmer (1994), where five searches are performed using 1,000 replicates with nearest neighbor interchange and MulTrees Off saving all shortest trees. The results of each of these searches are then used as the starting trees for a search using tree-bisection reconnection (TBR) and MulTrees on. The shortest trees from all searches were combined and a strict consensus was created. Bootstrap (BS) replicates were performed with 10,000 searches using the ''fast'' stepwise addition.

The partition homogeneity test (PHT; Farris et al. 1994) as implemented in PAUP* was performed on all data sets in this study. The test was carried out using 1,000 random partitions with indels rescored as multi-state characters and included in the partition to which they correspond. Searches used parsimony with simple taxon addition, TBR branch swapping and MulTrees on but limiting each search to one million topologies to minimize computer time. Because of the limitations on the searches, it is possible that the best topology for each data set was not found in each search, particularly in data sets that may have low support when analyzed alone. As a result, the PHT test, as implemented here, may be overly sensitive toward rejecting congruence since suboptimal trees may have been found for each partition. To account for this, bootstrap analyses (BS; Felsenstein 1985) were run on each of the data sets separately. Clades were considered incongruent between data only if they received >70% BS support. Clades which conflicted between data sets, but received <70% BS support were considered soft incongruence (Seelanen et al. 1997) and were not treated as a barrier to combining data. Based on these results the data were combined into a single data set and were used for all analyses. Maximum parsimony analyses were performed on the combined data set as described above for each data set individually.

Maximum likelihood analyses were performed using the general time-reversible (Yang 1994) model with some sites invariable and the remainder varying according to a gamma distribution (GTR + Γ+ I) as the best model for all data sets as determined by Modeltest (Posada and Crandall 1998). Parameters were estimated on a MP tree (single tree based on analysis with indels excluded) and were then fixed prior to beginning a simple addition sequence, TBR search with MulTrees on.

Bayesian analyses (BA) were conducted using MRBAYES version 3.0B4 (Huelsenbeck and Ronquist 2001). Analyses used four linked chains, heated sequentially with a heat of 0.2, run for two million generations and the GTR + Γ+ I model. All analyses were repeated a second time to confirm adequate mixing.

## RESULTS

Amplifications resulted in products ~400, ~300,~550, ~2,000, ~500, and ~750 bp each, for ITS, *trnL-trnF* spacer, G*CYC, ndhF, trnL* intron, and *rpl20-rps12,* respectively (Table 2). Amplification of *ncpGS* with the primers GS687f and GS856r resulted in two bands of ~500 and ~400 bp each for nearly all species sampled. These are referred to as *ncpGS1* and *ncpGS2* herein. The clones from each of these matched *ncpGS* genes in a BLAST search (Altschul et al. 1997) and since both were present for nearly all taxa, were treated as separate loci rather than alleles. Portions of the sequences from each locus were readily alignable and matched to exons but the intron regions were strongly divergent between loci and could not be aligned unambiguously. Previous work on this gene in Gesneriaceae has on occasions found duplications (Perret et al. 2003), but not as widespread as was found here. How-

TABLE 2. Nucleotide sequence characteristics of ITS, *trnL-trnF* spacer, GCYC, *ndhF, trnL* intron, *rpl12-rps20* spacer, *ncpGS1,* and *ncpGS2* for all species in the analysis. All data with gaps and missing characters excluded. Values of 0 for length and sequence divergence in parentheses indicate that this region was not amplified for all taxa in the analysis.

| Parameter | *ndhF* | ITS | *ncpGS1* | *ncpGS2* | *trnL* intron | *trnL-trnF* | GCYC | *rpl12-rps20* spacer |
|---|---|---|---|---|---|---|---|---|
| length range (bp) | 1542–2067 | 359–450 | (0)389–513 | (0)349–419 | 446–529 | 183–343 | 375–612 | 651–781 |
| length mean (bp) | 2004 | 433 | 487 | 388 | 500 | 320 | 565 | 747 |
| aligned length (bp) | 2072 | 503 | 522 | 419 | 552 | 364 | 632 | 833 |
| G/C content range (%) | 32.5–35.7 | 50.4–62.1 | 34.5–39.7 | 38.9–44.5 | 32.7–41.8 | 31.9–42.1 | 38.6–42.1 | 31.8–39.9 |
| G/C content mean (%) | 33.4 | 55.6 | 38.0 | 42.4 | 35.9 | 36.4 | 40.2 | 34.8 |
| number of indels | 2 | 34 | 14 | 5 | 7 | 13 | 10 | 5 |
| size of indels (bp) | 6–15 | 2–8 | 3–36 | 3–12 | 2–61 | 2–20 | 3–21 | 2–4 |
| % constant sites | 49.7 | 16.1 | 46.4 | 50.6 | 48.4 | 47.3 | 54.3 | 39.3 |
| % autapomorphic sites | 26.7 | 22.8 | 29.9 | 26.1 | 35.0 | 29.7 | 29.1 | 38.4 |
| % informative sites | 23.6 | 61.1 | 23.7 | 23.3 | 16.6 | 23.0 | 16.6 | 22.3 |
| % sequence divergence | 1.8–12.5 | 1.4–36.6 | (0)0.6–12.8 | (0)0.2–14.7 | 0.4–23.1 | 0.0–26.4 | 0.6–12.7 | 0.4–40.4 |

ever, Perret et al. (2003) used primer GS911r from Emshwiller and Doyle (1999) as their reverse primer rather than GS856r. Cloned sequences of *ncpGS* were nearly identical for all of the clones from the bands of different sizes, indicating that multiple alleles were not present for each of these bands. A few clones differed by a single to few nucleotides. These may represent different alleles, but the possibility that they are amplificition or sequencing errors can not be eliminated. Phylogenetic analyses that included these copies did not change the placement of the species they were isolated from. In a few cases either one of the bands was never recovered (*Gesneria christii*) or a sequence was recovered that was divergent to the point of not being alignable (*Gloxinia gymnostoma*), indicating that only a single locus, or other loci or alleles may be present in at least some taxa. Alternatively, it is possible that sequence divergence at the primer sites precluded amplification of the alternative locus. Cloned sequences of ITS resulted in sequences that were identical for each species and matched Gesneriaceae sequences in BLAST searches or, alternatively, matched a fungal contaminant in the BLAST search since the primers used were not plant specific. The fungal contaminants were obviously the explanation for the ambiguous sequences when these were sequenced without cloning.

The PHT detected a significant level of incongruence among all eight partitions ($p = 0.001$). However, due to the limitations placed on the searches for each of the data sets, it is unlikely that the best topology was found for all data sets. This is especially true for data sets where support is weak when analyzed separately such as the *trnL* intron and *trnL-trnF* spacer. Bootstrap analyses of each of the data sets did not yield any clades conflicting among analyses with BS support greater than 60% except for some interspecific relationships among tribe Gesnerieae (BS = 60–65%). Data were combined into a single analysis on the basis that the conflicts were more likely the result of soft incongruence (Seelanen et al. 1997) rather than true discrepancies.

Parsimony analyses of the combined data that included indels rescored as multi-state characters resulted in two trees of 7,076 steps each (Fig. 1; consistency index excluding uninformative characters (CI) = 0.44, retention index (RI) = 0.50). The only differences between the two MP trees with indels rescored are the relationships among *Capanea* and *Kohleria* species as indicated with an asterisk in Fig. 1. Analyses that excluded indels resulted in a single tree of 6,895 steps (CI = 0.43, RI = 0.49) and differed only in the placement of the *Eucodonia/Smithiantha* clade as sister to *Achimenes/Moussonia* with BS = 26%. The BS consensus placed the *Eucodonia/Smithiantha* clade sister to the *Achimenes/Moussonia* clade with BS = 87% and *Goyazia/Koellikeria* sister to clade C with *Niphaea/Solenopho-*

*ra* sister to clade C + *Goyazia/Koellikeria*. However, BS values were low (<30) for these latter relationships.

A ML analysis of the combined data excluding indels resulted in a single tree of score −48,592.745, alpha = 0.736. This tree (Fig. 2) is similar in topology to the MP trees, mainly differing in relationships that are only weakly supported in the MP analyses (Fig. 1). The *Eucodonia/Smithiantha* clade is placed sister to the *Achimenes/Moussonia* clade (Fig. 2) as was seen in the BS consensus with indels rescored. The BA trees did not differ between runs and only differed from the ML tree in that *Lembocarpus* and *Cremersia*, as well as *Gesneria christii* and *G. ventricosa* were sister to each other.

In the MP analysis of the combined data (Fig. 1) all tribes are strongly supported as monophyletic (BS 95–100%) and are recovered as monophyletic with strong support in the ML and BA analyses (Fig. 2). Within tribes Episcieae and Gesnerieae, for which generic sampling was only moderate for the former and species sampling moderate for the latter, relationships are moderate to well supported with BS ranging from 58–100%. Relationships within Gloxinieae receive a wide array of support, from essentially none to strong (>75%). Most of the data provide support toward the tips of the tree, linking together groups of genera in small, well-supported clades (BS 78–100%). A few other clades receive moderate support with BS ranging from 50–68%, whereas the remainder of relationships uniting these several clades are all weakly supported. The relationships among these clades often differ based on indel scoring and data analysis (MP or ML), but several weakly supported clades are recovered in all trees regardless of scoring and analysis.

*Gesneria* and *Rhytidophyllum* are each recovered as monophyletic contrary to previous analyses that had shown a monophyletic *Rhytidophyllum* embedded in a paraphyletic *Gesneria* (Zimmer et al. 2002). In this analysis both genera receive moderate to strong support as monophyletic (*Gesneria* at BS 71% and *Rhytidophyllum* at BS 97%; Fig. 1). Although each of the two genera are retained as monophyletic here, it should be emphasized that *Gesneria* comprises 46 species (Skog 1976) and the sampling here of five is likely to be inadequate to evaluate fully the separate monophyly of these two genera. Likewise the placement of *Bellonia* as sister to the monophyletic remainder of Gesnerieae receives only moderate support (BS 61%), although this relationship is recovered in all trees (Figs. 1, 2) and has been seen in analyses with smaller sampling (Smith et al. 2004). *Bellonia* bears a striking resemblance to several *Gesneria* species (such as *G. cubensis* (Decne.) Baill. and *G. pulverulenta* Liog.) in terms of vegetative morphology alone, but these species have not been included in any analyses to date and it is unresolved whether these vegetative similarities are synapomorphies or homoplasies due to a shared habitat.

*Phinaea* is clearly not monophyletic in these analyses and comprises two well-supported clades (Figs. 1, 2). The first of these clades is represented by two species, *P. divaricata* and *P. ecuadorana*, which are supported by BS of 100% and were recovered in nearly all data sets analyzed independently. These two species are remarkably similar both morphologically and with the sequence data sampled here and may not merit separate species status. Although their placement in clade A of Fig. 1 is only moderately to weakly supported with BS of 60%, this clade is recovered in all analyses of the combined data set (Figs. 1, 2).

***Sister Groups among Radial and Near Radial Flowered Genera.*** The goal of investigating the relationships of Gloxinieae and Gesnerieae in more detail was to resolve the sister group relationships of the genera with radial and near radial flowers. Although the relationships in parts of the tree are not yet strongly supported, the position of most taxa with radially or near radially symmetrical flowers are reasonably well-supported from these data. The position of *Bellonia* remains uncertain due to the possibility that greater sampling among species of *Gesneria* may show it to be contained within that genus. However, the current results of *Bellonia* as sister to the remainder of tribe Gesnerieae are consistent with chromosome counts ($n$ = 13 in *Bellonia* and $n$ = 14 in other Gesnerieae; Skog 1984). *Phinaea* is clearly not monophyletic and the exact placement of the two clades is uncertain. However, the placement of one group of species sister to the remainder of clade A (Figs. 1, 2) is consistent with other studies (Zimmer et al. 2002), is supported with BS of 60%, and was recovered in all trees with these data (Figs. 1, 2). The other group of species is less solidly supported as sister to *Monopyle* with BS of 56%, but again this relationship was found in all searches of these data. The placement of *Niphaea* as sister to *Solenophora* is solidly supported with BS of 95% and was recovered in all searches, although the exact placement of this clade remains uncertain.

***Molecular Evolution.*** To align the G*CYC* sequences analyzed here, 10 indels ranging from three to 21 base pairs were required, all in multiples of three. No stop-codons were detected in any of the sequences. A single copy of G*CYC* was found in all Gesnerioideae, as had previously been reported with a lesser degree of sampling (Möller et al. 1999; Citerne et al. 2000; Smith et al. 2004).

Tests for shifts in selection along specific lineages using PAML (Yang 2000) did not detect any significant differences along lineages leading to bilaterally or radially symmetrically flowered species (unpublished results). These results do not differ from a previous study that had less sampling within Gloxinieae and

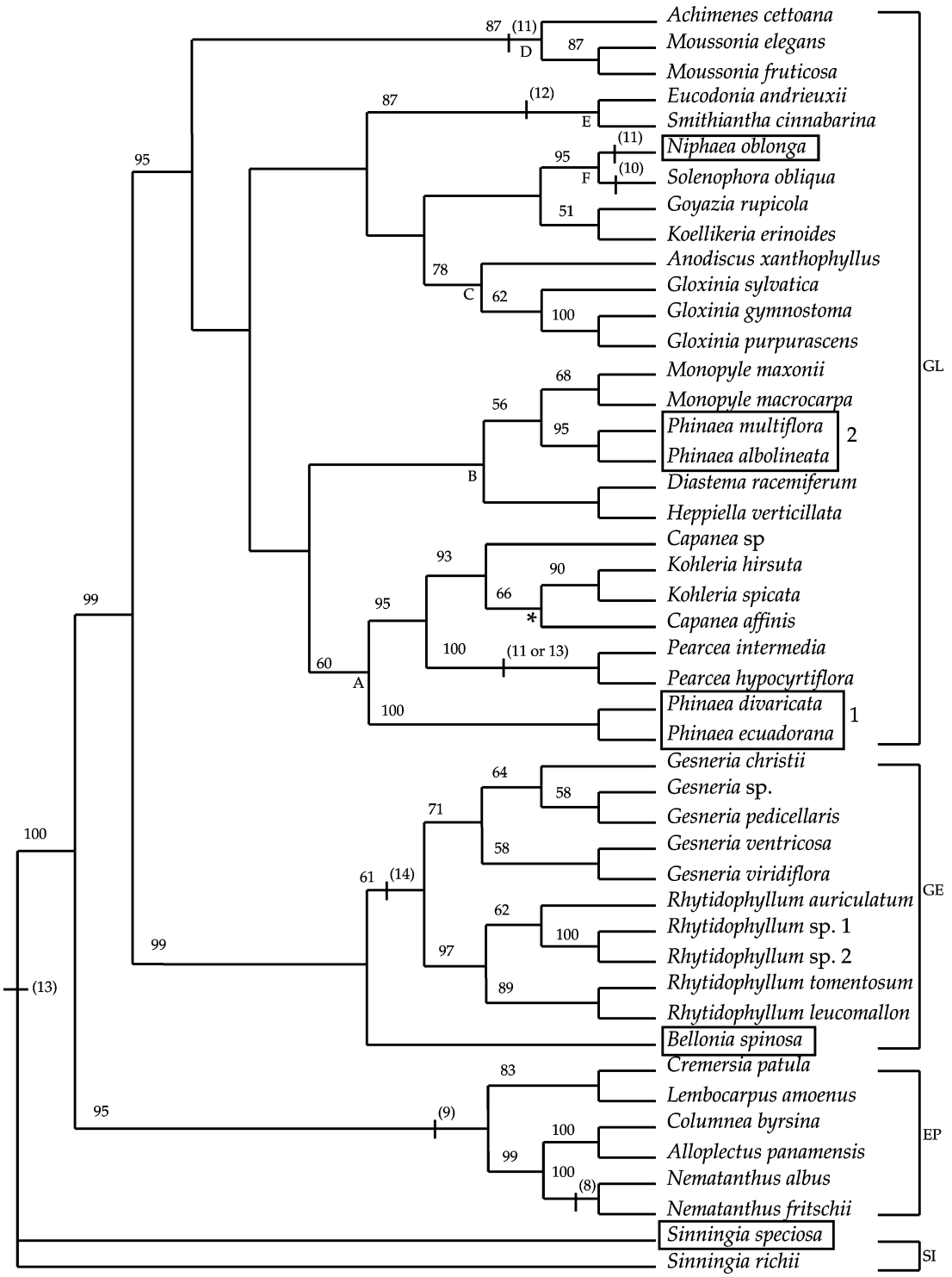


FIG. 1. One of two MP trees based on the combined data with indels rescored as multi-state characters (7,076 steps each, CI = 0.44, RI = 0.50). An asterisk marks the clade that collapses in the strict consensus. With indels excluded, one tree of 6,895 steps, (CI= 0.43, RI = 0.49) is recovered that differed only in the placement of the *Eucodonia/Smithiantha* clade as sister to *Achimenes/Moussonia* with a BS of 26%. Numbers above the clades are BS values and clades that lack BS values were not recovered in the BS consensus tree which differed in the placement of *Eucodonia/Smithiantha* as sister to *Achimenes/Moussonia* and *Goyazia/Koellikeria* as sister to clade C with *Niphaea/Solenophora* as sister to all of clade C including *Goyazia/Koellikeria*. Letters refer to clades discussed in the text. Boxes around taxon names represent species (or cultivated mutants in the case of

Gesnerieae and did not necessarily sample all sister group possibilities (Smith et al. 2004).

## Discussion

The data analysis including all sequences obtained in this study provides a reasonably well-supported tree for most relationships (Figs. 1, 2). Although the PHT demonstrated a significant level of incongruence among the eight data sets, the tree that results from the combined analysis has greater support for most clades than when each data set is analyzed singly. This implies that for the most part, data are congruent and support a common phylogenetic signal (Mason-Gamer and Kellogg 1996; Soltis et al. 1998; Smith 2000a). Each of the moderate to well-supported clades is discussed below.

***Clade A.*** The *Phinaea*1 clade is sister to a group of three genera, *Pearcea*, *Capanea*, and *Kohleria* that is strongly supported with BS of 95%, posterior probability (PP) of 99% and was often recovered in analyses of each of the data sets analyzed separately. Traditionally these three taxa have been considered similar and species often have been misplaced among each other (Kvist and Skog 1992, 1996 [and references therein]). Morphologically this clade, excluding *Phinaea*, is united by a nectary of five lobes, which is distinctive among other members of Gloxinieae that have an annular nectary or none. The five-lobed nectary also is found in some plants of *Goyazia*, rarely in *Solenophora*, and *Diastema* (Wiehler 1983). The lobes in *Diastema* are distinctly different than those of the genera in this clade by being elongated and nearly spatulate in shape. The placement of *Kohleria* imbedded within a paraphyletic *Capanea* is the most frequently recovered topology from these data, but this is not strongly supported and is due to the minimal sampling of two of three species of *Capanea* (there may be up to 11; Burtt and Wiehler 1995) and two out of ~20 species of *Kohleria*. It is premature to make any taxonomic decisions on the monophyly of these two genera at this time.

The placement of *Pearcea* in clade A also demonstrates convergence of chromosome numbers. Previous studies have reported a chromosome count of $n=13$ for this genus which is in accordance with other members of this clade and the majority of Gloxinieae (Wiehler 1983; Burtt and Wiehler 1995). More recent counts have been made on vouchered plant material of *P. abunda* and these have indicated $n=11$ (Kvist and Skog 1996) which would be convergent with *Achimenes*/*Moussonia* and *Niphaea*. The chromosomes were described as being small and difficult to count, therefore additional counts are needed, as Kvist and Skog (1996) commented, since this number is unusual for this group of species.

***Clade B.*** The remaining *Phinaea* species are phylogenetically distinct from the first two, but strongly supported as monophyletic with BS of 95% and PP of 99% (within clade B of Figs. 1 and 2). This clade is weakly supported as sister to *Monopyle* with BS of 56% (Fig. 1), but more strongly supported with PP of 99% (Fig. 2). The sister to these genera, a clade of *Diastema* and *Heppiella* is also only weakly supported as sister with BS of 12% (Fig. 1), but more strongly supported with PP of 96% (Fig. 2). This clade is recovered without change in topology in all analyses, therefore although the data are not strong, there appears to be no conflict among the analyses in supporting these relationships.

There are few morphological characters to support the relationships of the genera in clade B apart from autapomorphies that make their placement among other genera difficult. *Monopyle* is distinctive in having strongly anisophyllous leaves that sometimes appear alternate. Only some species of *Achimenes* have such strong anisophylly among Gloxinieae. Wiehler (1983) also reports the presence of an osmophore and a faint scent in the flowers of *Monopyle*, unusual traits for Gesneriaceae in general. *Diastema* has always been difficult to place and morphologically has a unique nectary structure among Gloxinieae as well as having a weakly bilobed stigma, which it shares with some species of *Achimenes*. *Heppiella* likewise has been difficult to place with other genera in that it has free anthers (whereas other genera have coherent anthers), and the filaments in *Heppiella* do not coil to pull the dehisced anthers away from the corolla opening as they do in other genera (Kvist 1990). Lastly, even without the apparent polyphyly of *Phinaea*, species in this genus have been difficult to place with other genera due to the shortened corolla tube and near to fully radially symmetrical corolla. Affinities between *Phinaea*, *Niphaea*, and *Bellonia* have been suggested due to the similarity of the corolla among these genera (Zhaoran and Skog 1990). However the differences in chromosome counts between *Phinaea* and *Niphaea* as well as the fully radially symmetrical flower of *Bellonia* (five, occasionally six fertile stamens corresponding to six petals) and their phylogenetic placement indicates that the corolla symmetry among these taxa is not homologous (Smith et al. 2004).

***Clade C.*** This clade is recovered in all analyses

←

*Sinningia speciosa*) with radially or near radially symmetrical flowers. Bars associated with numbers in parentheses mark changes in the base chromosome number ($x$) for the clade with the number in parentheses. Abbreviations to right of tree are tribes: EP—Episcieae, GE—Gesnerieae, GL—Gloxinieae, and SI—Sinningieae.

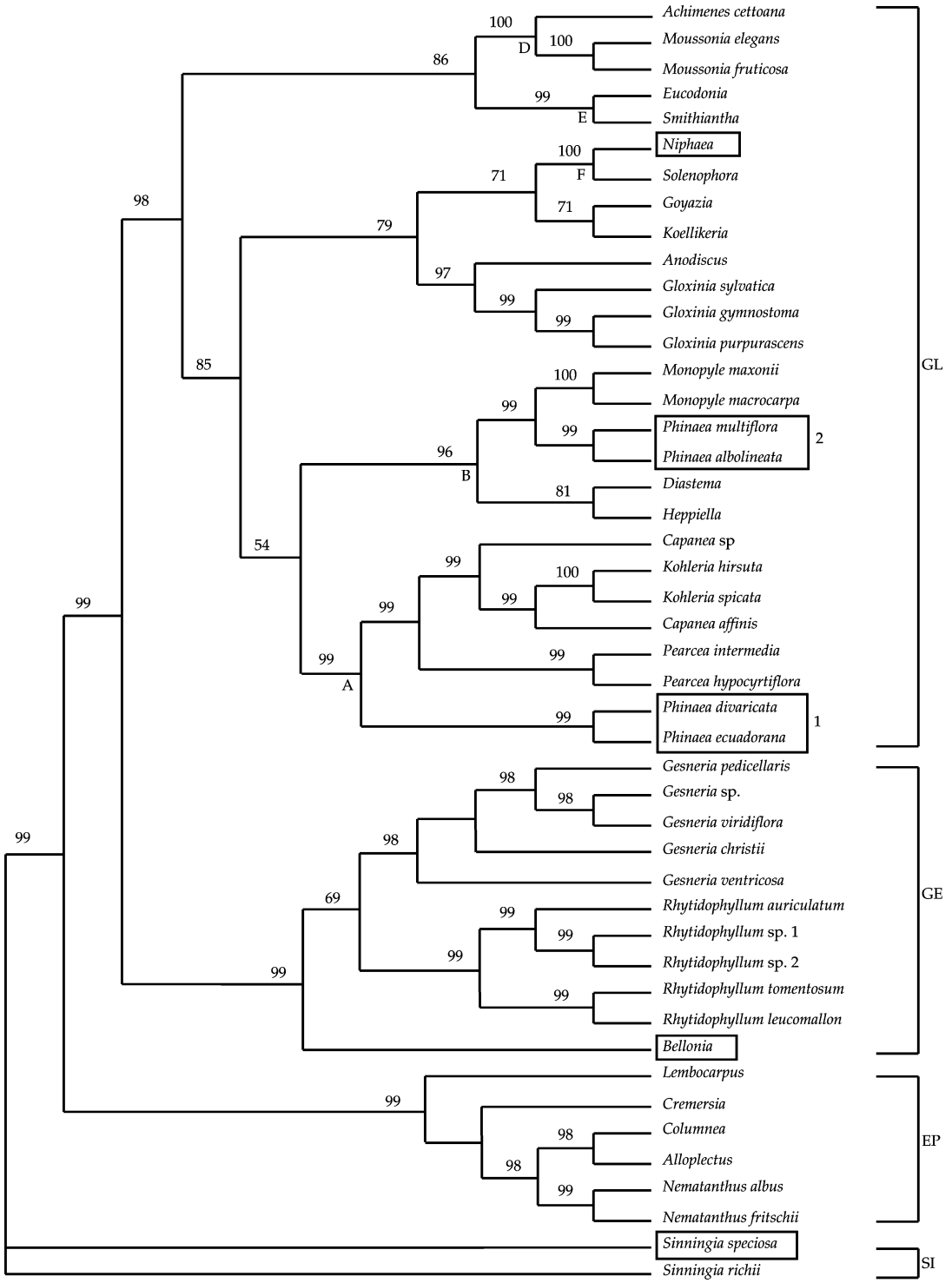


FIG. 2. ML and BA analysis of combined data with indels excluded. Boxes around taxon names represent species (or cultivated mutants in the case of *Sinningia speciosa*) with radially or near radially symmetrical flowers. Numbers above clades are Bayesian posterior probability values. Abbreviations to right of tree are tribes: EP—Episcieae, GE—Gesnerieae, GL—Gloxinieae, and SI—Sinningieae.

(Figs. 1, 2). The monophyly of *Gloxinia* is supported in this analysis with three species. *Gloxinia* has been shown to be polyphyletic based on other analyses (Zimmer et al. 2002), but the species sampling differed from the present study and the species that were divergent from the three sampled here were not included in this analysis. The species included here were initially described as species of *Seemannia* and sampling of *Seemannia* species and other *Gloxinia* species (*G. perennis* Fritsch) has indicated the genera may best be considered separate (Zimmer et al. 2002). One of the *Gloxinia* clades of Zimmer et al. (2002) coincides with the one here in that *G. purpurascens* was sampled in both and both analyses showed a close relationship between *Anodiscus* and *Gloxinia* (*Seemannia* of Zimmer et al. 2002). The close relationship of *Anodiscus* and *Gloxinia* is supported by the shared presence of a raceme-like inflorescence. The nectary is absent in *Anodiscus* and is either absent or a ring when present in *Gloxinia* (Wiehler 1983). Zimmer et al. (2002) also showed a close relationship between *Koellikeria* and *Gloxinia*. While *Koellikeria* is recovered in clades that encompass *Gloxinia* in all analyses here, it shows a closer relationship to the Brazilian narrow endemic *Goyazia* (Figs. 1, 2) that was not sampled in Zimmer et al. (2002). This smaller clade is sister to *Niphaea*/*Solenophora* in most analyses except the BS consensus tree where it was sister to clade C.

***Clades D-F.*** Three remaining clades in Gloxinieae are each well-supported (BS 87–95%; PP 99–100%) and vary in their relationships to each other depending on the analysis. These are *Achimenes*/*Moussonia* (D), *Eucodonia*/*Smithiantha* (E) and *Niphaea*/*Solenophora* (F). Support for the monophyly of each of these clades is in part provided by chromosome counts. While the remaining genera of Gloxinieae have $x = 13$ (except *Pearcea*), all of these genera represent different counts. *Achimenes* and *Moussonia* share a count of $x = 11$, which serves as an additional synapomorphy for this clade, but is homoplastic with *Niphaea* and *Pearcea*. Zimmer et al. (2002) did not show a sister group relationship between these two genera. The placment of *Moussonia* did not receive strong support in their analysis and a different species was used from the two included here. It is possible that *Moussonia* may not be a monophyletic genus. *Eucodonia* and *Smithiantha* are the only genera of Gesnerioideae known to have counts of $n = 12$, and *Solenophora* is unique among Gesnerioideae with a count of $n = 10$ (Skog 1984). These genera have a primarily, or exclusively, Mexican/Central American distribution, whereas the remainder of the tree is comprised of taxa with a largely South American distribution.

***Episcieae.*** Although Episcieae were included as an outgroup for this analysis and sampling encompassed only a few genera, there are some taxonomic differences pertaining to Gloxinieae that are reflected here. The first of these is the placement of *Lembocarpus* in Episcieae rather than Gloxinieae. *Lembocarpus* is highly reduced vegetatively, comprising a tuber that produces a single leaf and a single inflorescence with varying numbers of flowers seasonally. It is distributed in Surinam and French Guiana, where it grows on moss-covered rocks, generally in the proximity of streams where humidity is high (Leeuwenberg 1958). Due to the paucity of vegetative characters, the placement of *Lembocarpus* has been difficult. It has been traditionally placed in Gloxinieae (Wiehler 1983; Burtt and Wiehler 1995) on the basis of a tuber and annular nectary, which it shares with other Gloxinieae. However, it also has been placed in Episcieae where it shares characteristics with *Rhoogeton* such as superior ovary and tuberous habit (Leeuwenberg 1958). Unfortunately the placement of *Rhoogeton* also has been controversial, again largely due to its reduced vegetative body. Seed surface morphology shows a close affinity between *Lembocarpus* and *Episcia* (Beaufort-Murphy 1983), implying a placement in tribe Episcieae, but overall shape of the seed is similar to *Smithiantha* (Gloxinieae) and implies affinities for Gloxinieae. Smith (2001) placed *Lembocarpus* in Gloxinieae with a phylogenetic analysis of *ndhF* sequences. Fresh material of *Lembocarpus* was unavailable at that time and DNA from herbarium material was used to obtain partial sequences of *ndhF*. The sequences included in the present analysis were from freshly collected leaf tissue and the DNA posed no difficulties in amplification for the suite of DNA regions used here. The sequences were unambiguously aligned and indicate that previous assumptions regarding the placement of *Lembocarpus* in Gloxinieae (Wiehler 1983; Burtt and Wiehler 1995; Smith 2001) have been in error.

*Cremersia*, another narrowly endemic genus from French Guiana, is currently known only from the type locality and only recently described (Feuillet and Skog 2003). In this analysis, *Cremersia* is firmly placed in Episcieae. Although it was presumed that *Cremersia* would fall in this clade based on similar fruit structures that it shares with *Rhoogeton* (Feuillet and Skog 2003), phylogenetic analyses had not verified its placement in Episcieae prior to this analysis.

***Molecular Evolution.*** As with previous studies (Möller et al. 1999; Citerne et al. 2000; Smith et al. 2004), no mutations at the DNA level in G*CYC* can explain the differences in naturally occurring floral symmetry seen in Gloxinieae and Gesnerieae. The sequences examined here had indels, but always in multiples of three and no premature stop codons were detected in any of the sequences. Molecular evolutionary analyses of G*CYC* sequences across Gesneriaceae suggest that purifying selection is acting to a similar extent along lineages leading to bilaterally symmetrical and

radially symmetrical taxa (Smith et al. 2004). Thus, there is no evidence that functional changes in *GCYC* are correlated with shifts away from bilateral symmetry. It is possible that functional shifts in *GCYC*, as measured by changes in the rate ratio of non-synonymous to synonymous substitution, have gone undetected by the methods implemented here (unpublished results) and previously (Smith et al. 2004). However, it is more likely that changes in *GCYC* expression, and not function, are responsible for the evolution of radially symmetrical flowers in Gesneriaceae. It is also possible that changes in either function or expression of genes downstream of *GCYC*, or in independent genetic pathways, affected shifts in floral symmetry in Gesneriaceae. Alternatively, as *GCYC* has not been demonstrated to regulate floral symmetry in Gesneriaceae, and its function only inferred from a relatively close phylogenetic relationship with *Antirrhinum*, *GCYC* may not be involved in controlling floral symmetry in Gesneriaceae. Further functional and expressional studies are need to address the role of *GCYC* in the evolution of flower morphology in Gesneriaceae.

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