MOLECULAR PHYLOGENETICS

Untangling Gloxinieae (Gesneriaceae). I. Phylogenetic patterns and generic boundaries inferred from nuclear, chloroplast, and morphological cladistic datasets

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Tribe Gloxinieae has been estimated to include 22 genera and approximately 290 species. This study presents maximum parsimony phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer regions sequences, the chloroplast DNA *trnL* intron and *trnL-trnF* intergenic spacer region sequences, a morphological cladistic dataset, and combined analyses of these datasets. These analyses suggest that the genera *Gloxinia*, *Phinaea*, and (possibly) *Diastema* are polyphyletic; *Kohleria* is paraphyletic in relation to *Capanea*; *Bellonia* and *Pheidonocarpa* should be considered members of tribe Gesnerieae; and *Lembocarpus* is a member of tribe Episcieae. Furthermore, the historically recognized genus *Seemannia*, now included in *Gloxinia*, appears to form a strongly supported monophyletic group; several *Gloxinia* species from southern Brazil appear to be most closely related to *Goyazia*; *Capanea*, *Kohleria*, *Pearcea* s.l., and *Diastema vexans* appear to form a strongly supported clade; and *Diastema*, *Monopyle*, *Phinaea* (in part), and a few *Gloxinia* species (*Gloxinia dodsonii*, *G. lindeniana*, and *G. racemosa*) form a clade. Classification issues and generic boundaries of these lineages are discussed in detail.

KEYWORDS: generic boundaries, Gesneriaceae, Gesnerieae, Gloxinieae, ITS, molecular phylogenetics, morphology, *trnL-F*.

INTRODUCTION

Gesneriaceae are well known for their diversity of floral and vegetative form (Wiehler, 1983; Harrison & al., 1999; Möller & Cronk, 2001; Zimmer & al., 2002; Roalson & al., 2003). In addition, Gesneriaceae have been plagued by continual questions of taxon circumscription, with different interpretations of the morphological circumscription of tribes, genera, and species almost as common as the number of workers in the field (e.g., Wiehler, 1983; Skog & Kvist, 2000). A particularly tenacious problem has been generic circumscription—many species have been described under four or more different generic names (i.e., *Kohleria tubiflora* has been classified as *Brachyloma*, *Cryptoloma*, *Gesneria*, *Isoloma*, and *Kohleria*; Kvist & Skog, 1992). Moore & Lee (1968) aptly stated "...it is sometimes easier to distinguish species in the Gesneriaceae than it is to place them properly to genus". One potential explanation of this confusion of relationships in the family is that morphological convergence and parallelisms have occurred across the family, particularly in characters such as floral form, leading to confusion in what morphological characteristics are useful in understanding evolutionary relationships, and which may be homoplastic in relation to phylogeny.

Molecular phylogenetic techniques have been found to be extremely useful in exploring phylogenetic relationships, teasing apart morphological similarity due to phylogenetic relatedness or convergence, and identifying and sorting out taxa that have been based on plesiomorphic characters in Gesneriaceae (Harrison & al., 1999; Möller & Cronk, 2001; Zimmer & al., 2002; Mayer & al., 2003; Perret & al., 2003; Roalson & al., 2003), as well as other plant groups (Convolvulaceae: Manos & al., 2001; Crassulaceae: Mort & al., 2002; Orchidaceae: Goldman & al., 2001; Themidaceae: Pires & Sytsma, 2002). Here we explore phylogenetic relationships and generic boundaries in tribe Gloxinieae using nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences, chloroplast tRNA intron and intergenic spacer (*trnL-F*) sequences, and a morphological cladistic dataset.

Tribe Gloxinieae includes 22 genera and approxi-

mately 290 species as currently circumscribed (Burtt & Wiehler, 1995). Recent molecular phylogenetic studies have suggested that a portion of the tribe (the genera *Paliavana*, *Sinningia*, and *Vanhouttea*) are not closely related to the rest of Gloxinieae and would be best dealt with as a separate tribe Sinningieae (Smith & al., 1997; Zimmer & al., 2002), as it was treated by Fritsch (1893–1894). On the basis of these studies, Weber (2004) has formally segregated Sinningieae from Gloxinieae. Other species (e.g., *Gloxinia sarmentiana*) also appear to not belong to Gloxinieae sensu Zimmer & al. (2002), although their true affinities are unclear. Recircumscribed Gloxinieae include approximately 19 genera and 215 species sensu Burtt and Wiehler (1995), although some have suggested the total mumber is fewer (17 genera and 172 spp.; Weber, 2004). Examples of several genera are presented in Fig. 1A–E.

Gloxinieae include a broad array of vegetative, floral, nectary, and fruiting forms. While most of the genera of Gloxinieae are herbaceous with scaly rhizomes, *Solenophora* includes woody species that sometimes become small trees (Weigend & Förther, 2002). The majority of species in the tribe have zygomorphic flowers that are salverform, funnelform, or tubular, but three genera have almost rotate corollas: *Bellonia*, *Niphaea*, and *Phinaea* (Wiehler, 1983). Nearly every possible corolla color is represented within the tribe: pink, purple, red, white, yellow, blue, orange, and various combinations of these on the same corolla. Nectaries are organized in numerous configurations, including separate glands, annular rings, or completely absent. Fruits can be variously structured with fleshy or dry capsules (for overview, see Wiehler, 1983). Gloxinieae include species ranging from central Mexico to southern Brazil and most of the islands of the Caribbean with chromosome complements of $x = 10$, 11, 12, and 13.

While the last 10 years have seen several phylogenetic studies dealing with relationships in Gloxinieae, no clear picture of generic relationships has resulted (Smith, 1996; Smith & Carroll, 1997; Smith & al., 1997; Smith & Atkinson, 1998; Smith, 2000, 2001; Zimmer & al., 2002). Until recently the relationship of Gloxinieae to Gesnerieae has been unclear, with several studies suggesting Gesnerieae to be nested within Gloxinieae (Smith & Atkinson, 1998; Smith, 2000, 2001). The study of Zimmer & al. (2002) has given strong support to the sister clade relationship of these tribes when excluding *Sinningia*, *Paliavana*, and *Vanhouttea*. However, since all genera have not yet been sampled, all generic affinities are not yet clear. In regards to generic boundaries in Gloxinieae, Zimmer & al. (2002) suggested that the current definition of one genus circumscribes a polyphyletic group (i.e., *Gloxinia*).

This lack of resolution of generic relationships, the questionable nature of some generic boundaries, and the historical variability of generic circumscription in this tribe highlights the need for a more in-depth study of these relationships and boundaries. In this study we use a morphological cladistic dataset, nuclear ribosomal DNA internal transcribed spacer (ITS) sequences, and chloroplast DNA *trnL-F* intron-spacer sequences to address five phylogenetic and classification questions: (1) Do nuclear, chloroplast, and morphological cladistic datasets suggest the same phylogenetic relationships in Gloxinieae? (2) What is the consensus phylogenetic pattern found in Gloxinieae? (3) What genera belong to the clade/tribe Gloxinieae? (4) What are the relationships among genera of the tribe? (5) What is the relationship between generic boundaries and phylogenetic relationships? We also directly test Wiehler's (1983) circumscription of tribe Gloxinieae, Wiehler's numerous (re-) circumscriptions of several genera (Wiehler, 1975a, 1976, 1983), Wiehler's (1978) creation of the genus *Parakohleria* and Kvist and Skog's union of *Parakohleria* with *Pearcea* (Kvist & Skog, 1996).

MATERIALS AND METHODS

Sampling. — Samples were selected from live plants grown at the Smithsonian National Museum of Natural History Botany Research Greenhouses, herbarium specimens at US, and silica-dried leaved available from John L. Clark (Appendix 1). The DNAs of 44 accessions/taxa were added to the previously sampled 48 accessions/taxa documented in Zimmer & al. (2002). The two *Solenophora* samples were obtained from H. Förther (Institut für Systematische Botanik der Universität München, Germany). We have sampled here at two levels. First, a few samples from each of the major clades of Gesneriaceae subfamily Gesnerioideae were analyzed with samples from genera that have uncertain tribal placement, using two samples from outside Gesnerioideae as outgroups (Zimmer & al., 2002). This included samples of all five Gesnerioideae tribes, 36 genera, and 44 species. The genera of uncertain tribal placement analyzed here include *Bellonia*, *Lembocarpus*, and *Pheidonocarpa*. Second, as many species and genera as possible of Gesneriaceae tribe Gloxinieae were included in Gloxinieae molecular analyses based on recent collections or live material. Outgroups were chosen from tribe Gesnerieae based on previous analyses (Zimmer & al., 2002; outgroup analyses presented here). Some species were not sampled due to lack of recent collections from which to sample DNA. The only genus not sampled extensively was *Achimenes*, since recent analyses have already addressed relationships within the genus (Roalson & al., 2003). Rather, three samples representing

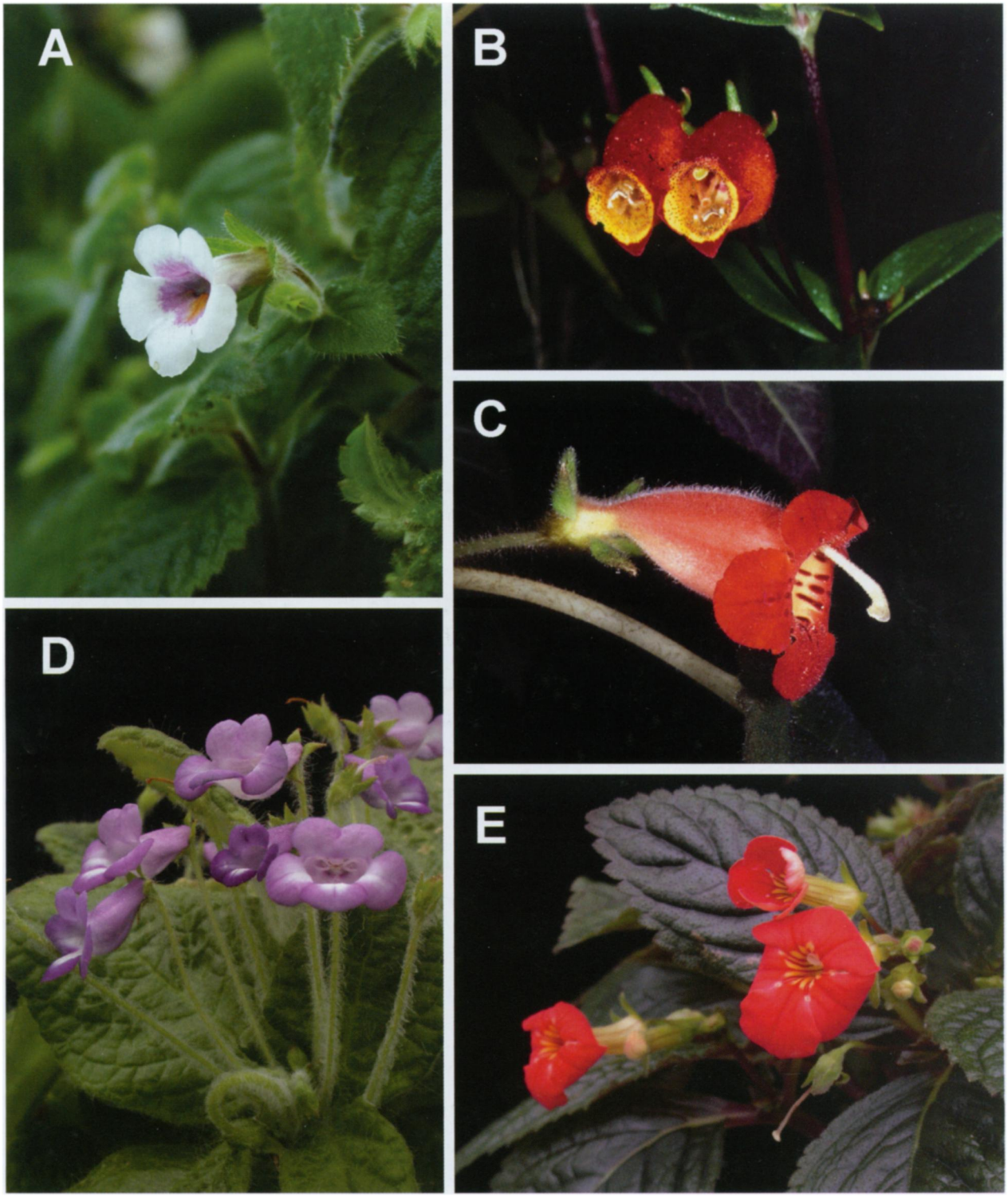

Fig. 1. Exemplar taxa of the Gloxinieae tribe. A, *Achimenes misera* (photo by John R. Clark); B, *Gloxinia sylvatica* (photo by Eric H. Roalson); C, *Kohleria grandiflora* (photo by Eric H. Roalson); D, *Eucodonia andrieuxii* (photo by Leslie Brothers; © Smithsonian Institution); E, *Diastema comiferum* (photo by Leslie Brothers; © Smithsonian Institution).

the three main clades of *Achimenes* identified in that study are used to represent the lineage. There is incomplete overlap of the ITS and *trnL-F* datasets as the *trnL-F* region was not available for all samples present in the ITS dataset. In comparison to the ITS dataset, one *trnL-F* sequence is completely missing, three sequences are

missing the *trnL* intron, and three sequences are missing the *trnL-F* intergenic spacer. These species were excluded from the *trnL-F* dataset analysis. Finally, the Gloxinieae morphological dataset included species where good descriptions and specimens were available, including some species not included in the molecular dataset.

DNA sequencing. — DNA was isolated using the Quiagen DNeasy DNA isolation kit or traditional CTAB extraction protocols (Doyle & Doyle, 1987). Templates of the nrDNA internal transcribed spacer region (ITS) were prepared using the primers ITS5HP (Suh & al., 1993) and ITS4 (White & al., 1990). The chloroplast spacer regions were amplified using the primers *trnL*c and *trnL*f for the *trnL* intron and *trnL-trnF* intergenic spacer (igs; Taberlet & al., 1991).

For some herbarium material it was necessary to amplify and sequence the ITS1, ITS2, *trnL* intron, and *trnL-F* intergenic spacer as individual units. The ITS1 spacer was amplified with primers ITS5HP and ITS2 (White & al., 1990), the ITS2 spacer was amplified with primers ITS4 and ITS3 (White & al., 1990), the *trnL* intron was amplified with *trnL*c and *trnL*d (Taberlet & al., 1991), and the *trnL-F* igs was amplified with *trnL*f and *trnL*e (Taberlet & al., 1991). Polymerase chain-reaction (PCR) amplifications followed standard procedures described by Zimmer & al. (2002) utilizing Taq DNA polymerase (Promega) and Mg HotBead (3.0 mM; Lumitekk).

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 the PEG precipitation procedure (Johnson & 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). Sequencing of the ITS region utilized the primers ITS5HP and ITS4 for the entire ITS region, or ITS5 and ITS2 for the ITS1 spacer and ITS4 and ITS3 for the ITS2 spacer, when these two regions were amplified separately. Sequencing of the chloroplast spacers made use of *trnL*c and *trnL*f, or *trnL*c and *trnL*d for the *trnL* intron and *trnL*e and *trnL*f primers for the *trnL-F* igs. Sequencing was performed using an Applied Biosystems Model 377 Automated DNA Sequencing System.

Automated DNA sequencing chromatograms were edited, and contigs were assembled using Sequencher 3.0 or 4.1 (Gene Codes Corporation, Inc.). The sequences were truncated to include only ITS1, 5.8S, ITS2, the *trnL* intron, the *trnL* exon 2, and the *trnL-trnF* igs regions. Identification of the ends of ITS1, ITS2, and the ends of the chloroplast spacers were determined by comparisons with other Gesneriaceae sequences (Zimmer & al., 2002). All sequences were manually aligned. Gaps have not been coded as separate characters in any of the following analyses due to the complex and/or overlapping nature of many of the gaps in this dataset. Additionally, missing data for some taxa in combined analyses would make accurate gap coding difficult. Newly acquired sequences were deposited in GenBank (accessions AY702350 to AY702436).

Morphological dataset. — Characters and character states were determined by examination of living material, by examination of herbarium specimens, and from the literature (Skog, 1976; Wiehler, 1976, 1978, 1983; Beaufort-Murphy, 1983; Kvist, 1990; Xu & Skog, 1990; Kvist & Skog, 1992, 1996; Weigend & Förther, 2002). Character states based on the literature were verified by examination of live and/or herbarium material to the greatest extent possible. Emphasis was placed on characters used to define taxa at the tribal and generic levels in recent classifications.

With few exceptions, all taxa included in the molecular analyses were also included in the morphological analysis. Several species had to be scored with partial data because certain characters (in particular, chromosome numbers and fruit and seed characters) are not described in the literature and herbarium, and live material were insufficient for scoring these characters. Taxa missing such data are *Achimenes glabrata*, *Diastema comiferum*, and *Goyazia rupicola* (see Appendices 2 and 3). For this reason additional species were included in the morphological analysis due to better knowledge of such characters, allowing more complete scoring of species closely related to those included in the molecular analysis that were missing certain morphological data (complete list of species included listed in Appendix 3).

Definition and scoring of morphological characters for cladistic analyses is fraught with difficulties and there are numerous different perspectives on the best way to code these characters (for a review, see Stevens, 1991). Particularly at issue is where the break points are among character states. An additional problem is that characters considered to be qualitative often prove to be quantitative in nature when explored in detail (Felsenstein, 1988; Stevens, 1991).

Organization of character states within characters took advantage of natural breakpoints in character state distributions. Those characters that could not be organized in this fashion were excluded from the analyses. Most characters in the dataset presented here are relatively clear presence/absence characters. Those that are multistate rather than presence/absence characters have clear character states and were nearly always easy to define and score. Most potential characters that deal with near continuous variation, particularly associated with

dimension measurements, were excluded as has been suggested previously (Cranston & Humphries, 1988). The exceptions to this (characters 11, 58, and 61) have either clear-cut discontinuities, or are important characteristics for defining particular groups and appear to be clear and consistent, at least for some lineages. In particular this applies to character #11: leaf vein number (Appendix 2). This character is surprisingly consistent within a taxon, and most exceptions are widespread and variable taxa, some of which may be overlumped.

Sixty-two characters were investigated and used in the analysis (Appendix 2). Thirty-two of the characters are associated with flowers and fruits, while the other thirty characterize vegetative, habitat, chromosome, distribution, and other miscellaneous aspects of species in Gloxinieae. One hundred and eleven cells (2.1%) of the data matrix are scored as missing data (Appendix 3).

Maximum parsimony analysis of molecular data from subfamily Gesnerioideae. — Maximum parsimony (MP) analysis was performed using PAUP* 4.0b10 (Swofford, 2002). The analysis used heuristic searches (ACCTRAN; 500 random addition cycles; TBR branch swapping; STEEPEST DESCENT; MULTREES option in effect). Clade robustness was estimated using 500 heuristic bootstrap replicates (10 random addition cycles with 10 trees saved per cycle, TBR branch swapping; STEEPEST DESCENT; MULTREES option in effect; Felstenstein, 1985; Hillis & Bull, 1993). Three ingroup datasets were analyzed and compared: ITS, *trnL-F*, and a combined ITS/*trnL-F* dataset.

Homogeneity of the ITS and *trnL-F* datasets was assessed using the partition homogeneity test (Farris & al., 1995) as implemented in PAUP*4.0b10. Twenty thousand replicate data partitions were run (heuristic search; simple addition; no branch swapping), excluding constant characters. This test measures character congruence by comparing tree length differences among trees derived from resampled data partitions of the combined datasets and trees derived from the defined data partition.

MP analysis of tribe Gloxinieae. — Maximum parsimony (MP) analysis of the Gloxinieae molecular datasets used heuristic searches (ACCTRAN; 100 random addition cycles; TBR branch swapping; STEEPEST DESCENT; MULTREES option in effect). Clade robustness was estimated using the 100 heuristic bootstrap replicates (10 random addition cycles with 10 trees saved per cycle, TBR banch swapping; STEEPEST DESCENT; MULTREES option in effect; Felsenstein, 1985; Hillis & Bull, 1993). Due to the large number of potentially most parsimonious trees, the number of trees saved during each random addition replicate was limited to 500. Seven ingroup datasets were analyzed and compared: ITS, *trnL-F*, morphology, each pairwise combination of these, and all three datasets together. In all of the combined analyses that include the morphology character matrix, the morphological characters are equally weighted. The analyses combining morphology with individual or both molecular datasets include only a subset of the samples from either morphological or molecular datasets, as there is incomplete overlap.

Homogeneity of the ITS, *trnL-F*, and morphology datasets was assessed using the partition homogeneity test (Farris & al., 1995) as implemented in PAUP* 4.0b10. Twenty-thousand replicate data partitions were run (heuristic search; simple addition; no branch swapping), excluding constant characters.

Morphological MP analysis of tribe Gloxinieae. — Maximum parsimony (MP) analysis of the Gloxinieae morphological dataset used heuristic searches (ACCTRAN; 100 random addition cycles; TBR branch swapping; STEEPEST DESCENT; MULTREES option in effect). Due to the large number of potentially most parsimonious trees, the number of trees saved during each random addition replicate was limited to 500. Clade robustness was estimated using 100 heuristic bootstrap replicates (10 random addition cycles with 10 trees saved per cycle, TBR banch swapping; STEEPEST DESCENT; MULTREES option in effect; Felstenstein, 1985; Hillis & Bull, 1993).

In order to test the effect of equal weighting of all morphological characters on tree topology, alternative weighting schemes were assessed for the morphological cladistic dataset. Four weighting schemes were compared to equal weighting: (1) all characters associated with flower and inflorescence morphology were downweighted to half of the weight of the other characters; (2) all characters associated with fruit and seed morphology were downweighted to half of the weight of the other characters; (3) all characters associated with leaf and stem morphology were downweighted to half of the weight of the other characters; and (4) all characters associated with indument characters were downweighted to half of the weight of the other characters. The purpose of testing differential weighting of the different morphological character sets is to assess whether particular sets of characters, such as characters of floral form, are biasing the results of the rest of the morphological dataset.

RESULTS

MP analysis of molecular data from subfamily Gesnerioideae. — Maximum parsimony analysis of the ITS/*trnL-F* outgroup dataset resulted in 144 most-parsimonious trees (length = 1958 steps, CI [consistency index; Kluge & Farris, 1969] = 0.502, RI [retention index; Farris, 1989] = 0.615, RC [rescaled consistency index; Farris, 1989] = 0.309). Figure 2 is the strict con-

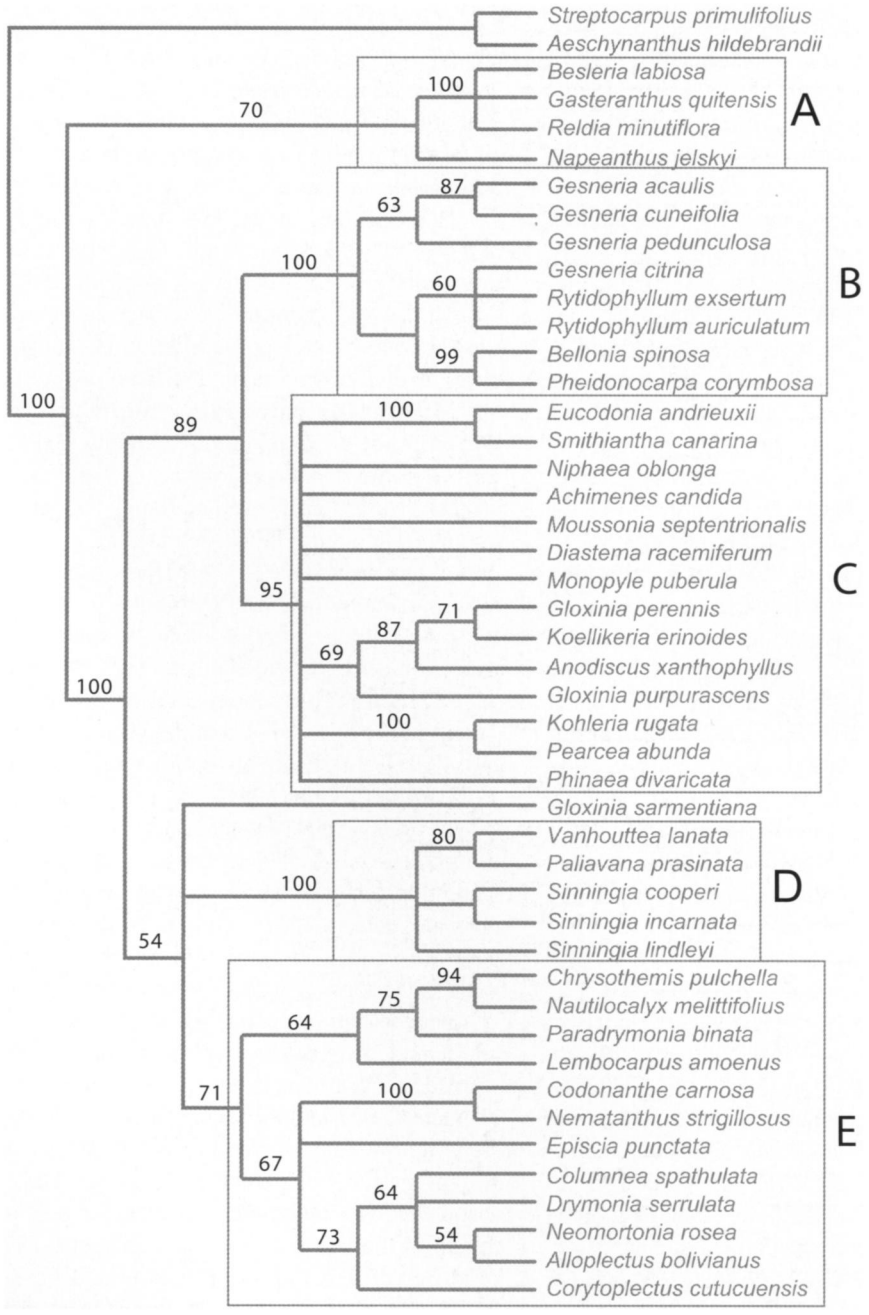

Fig. 2. Analysis of relationships within Gesnerioideae using the nrDNA ITS and cpDNA *trnL-F* datasets. Strict (MP) consensus tree of 144 most-parsimonious trees of 1958 steps. Numbers above branches are bootstrap percentages where branch support is equal to or greater than 50%. Shaded boxes refer to the following tribal-level clades: A, Beslerieae/Napeantheae clade; B, Gesnerieae clade; C, Gloxinieae clade; D, Sinningieae clade; and E, Episcieae clade.

sensus of these trees. Here, only the combined ITS/*trnL-F* outgroup results are presented, as the partition homogeneity test suggests that there is no significant difference between the ITS/*trnL-F* partition and random partitions (P = 0.4113). The majority of the samples in this analysis were previously published (Zimmer & al., 2002), and therefore further details of this dataset not associated with the new sequences will not be presented.

The primary purpose of this analysis was to address the phylogenetic affinities of *Bellonia*, *Lembocarpus*, and *Pheidonocarpa*. *Bellonia* and *Pheidonocarpa* are strongly supported as belonging to tribe Gesnerieae (bootstrap [bs] = 100%). *Lembocarpus* appears to be a member of tribe Episcieae (bs = 71%). *Lembocarpus* is not included in further analyses as we are not here addressing relationships in Episcieae, but *Bellonia* and *Pheidonocarpa*

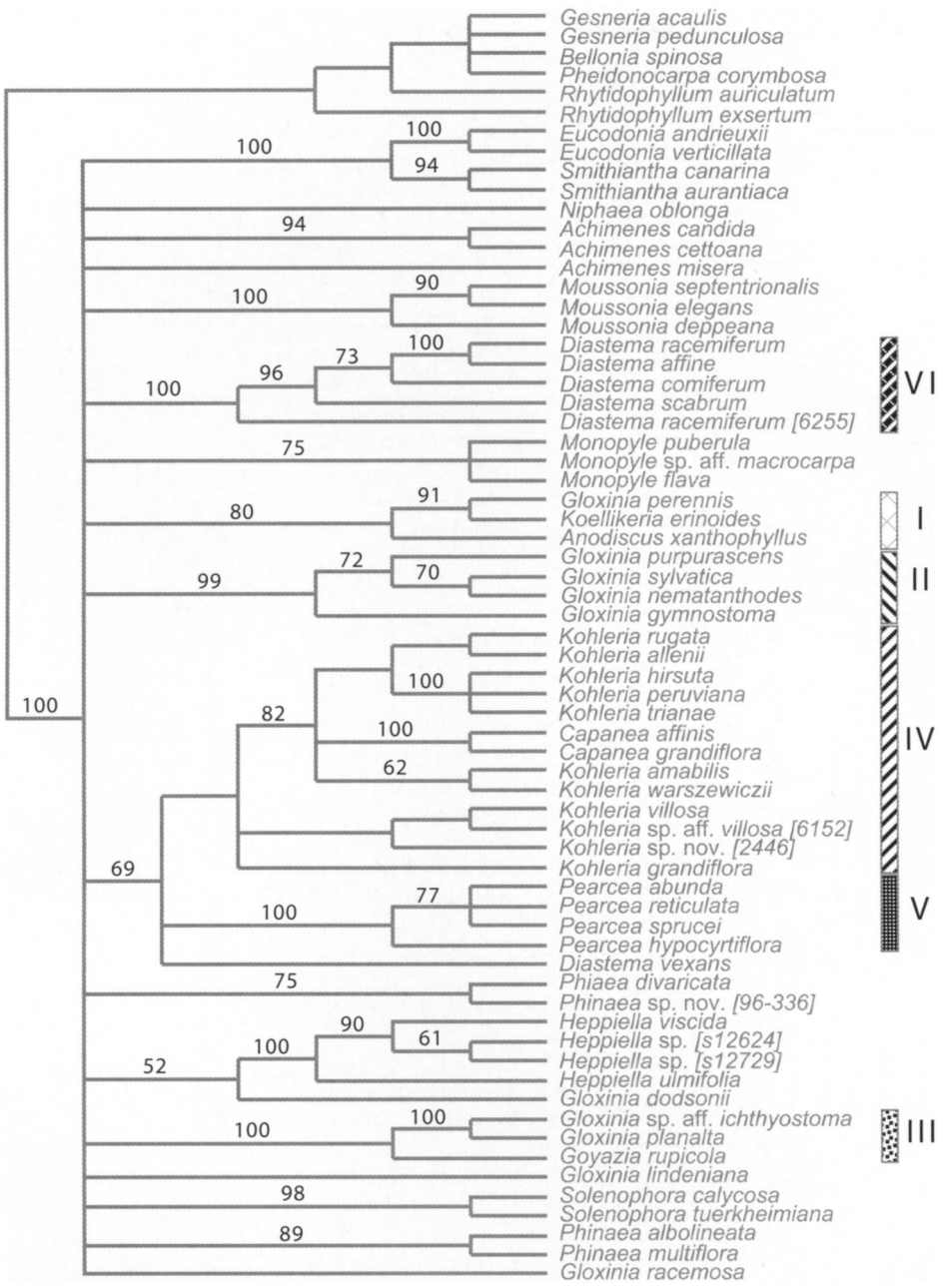

Fig. 3. Analysis of relationships within Gloxinieae using the nrDNA ITS dataset. Strict (MP) consensus tree of 24000 most-parsimonious trees of 1057 steps. Numbers above branches are bootstrap percentages where branch support is equal to or greater than 50%. Major well-supported clades from the combined analyses and discussed in the text are labeled: I, *Gloxinia*-type clade; II, *Seemannia* clade; III, Brazil clade; IV, *Kohleria* clade; V, *Pearcea* clade; VI, *Diastema* clade.

samples are included with *Gesneria* and *Rhytidophyllum* as outgroup taxa in further analyses.

Sequencing and alignment characteristics for tribe Gloxinieae. — The two or 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 653 bp (base pairs) long with 323 variable sites (49.5%), of which 229 (35.1%) were parsimony informative. The length of the unaligned complete sequences varied from 609 to 623 bp. One sequence is missing a portion (57 aligned bp) of the 5' end of the ITS1 spacer, and three sequences are missing a portion (2–17 aligned bp) of the 3' end of the ITS 2 spacer due to poor sequencing reads of these regions. Additionally, six sequences are missing a portion (13–72 bp) of the 5.8S gene due to incomplete

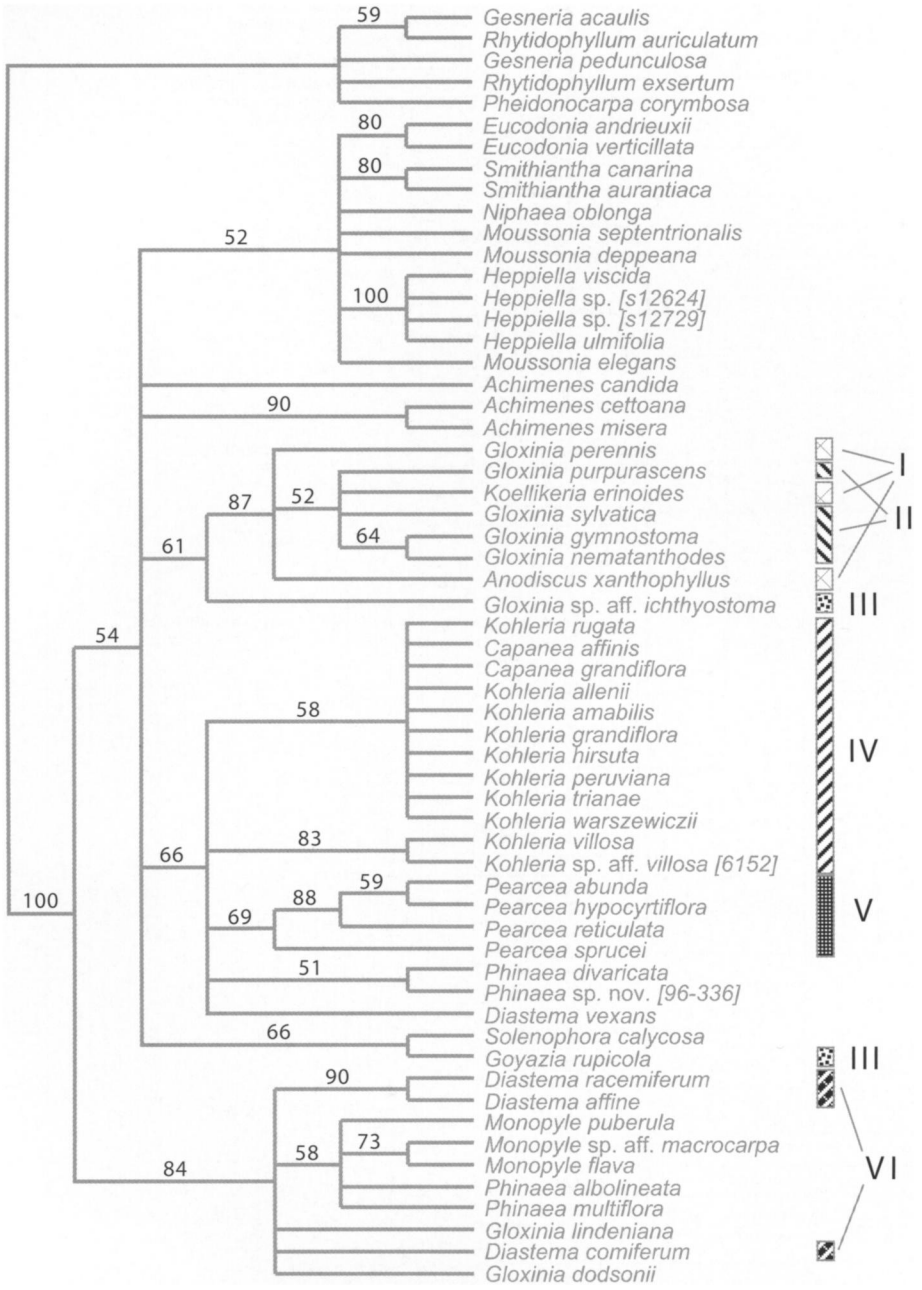

Fig. 4. Analysis of relationships within Gloxinieae using the cpDNA *trnL-F* dataset. Strict (MP) consensus tree of 128 most-parsimonious trees of 171 steps. Numbers above branches are bootstrap percentages where branch support is equal to or greater than 50%. Major well-supported clades from the combined analyses and discussed in the text are labeled: I. *Gloxinia*-type clade; II, *Seemannia* clade; III, Brazil clade; IV, *Kohleria* clade; V, *Pearcea* clade; VI, *Diastema* clade.

overlap of separate sequencing of the ITS 1 and ITS 2 spacers. The alignment resulted in 59 gaps ranging from 1 to 8 bp in length. This data alignment resulted in uncorrected pairwise sequence divergence within the ingroup of 0–16%.

The two or four *trnL-F* sequencing primers produced overlapping fragments that collectively covered the entire *trnL* intron, *trnL* exon 2, and the *trnL-F* intergenic spacer along both strands. The aligned *trnL-F* data matrix was 944 bp long with 139 variable sites (14.7%), of which 52 (5.5%) were parsimony informative. The length of the unaligned complete sequences varied from 880 to 908 bp for the *trnL-F* sequences. Fourteen sequences are missing a portion (9–35 bp) of the 5' end

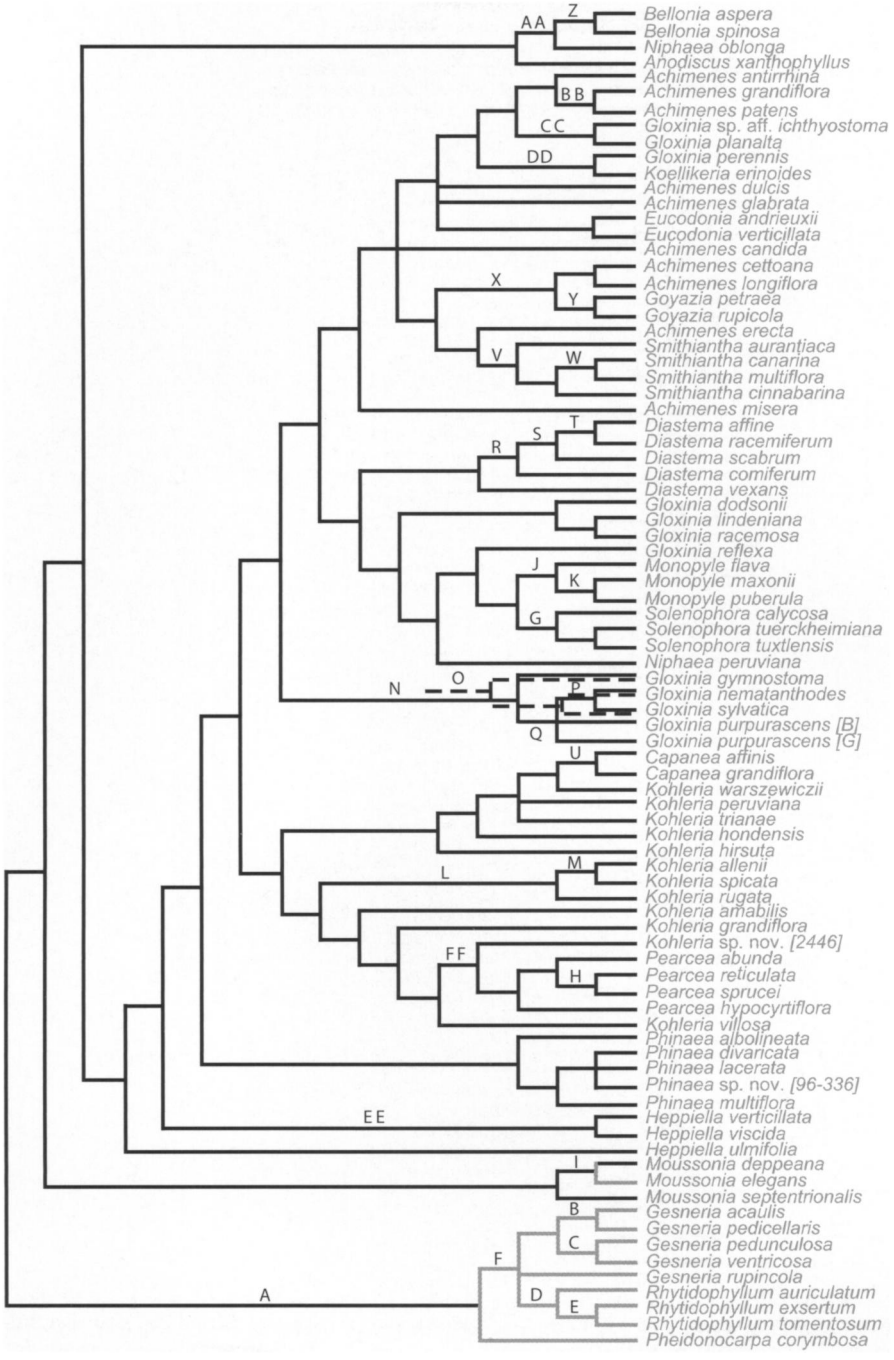

Fig. 5. Analysis of relationships within Gloxinieae using the morphological (fruit and seed characteristics down-weighted by half) dataset. Strict (MP) consensus tree of 47 most-parsimonious trees of 388 steps. Letters above branches refer to branch support under different weighting schemes where bootstrap values are equal to or greater than 50%, listed in Appendix 4.

of the *trnL* intron and one sequence is missing 77 bp of the *trnL* intron (3' end)/*trnL* exon 2/*trnL-F* igs (5' end) region due to incomplete sequence overlap of that region. The alignment resulted in 37 gaps ranging from 1 to 23 bp in length. This data alignment resulted in uncorrected pairwise sequence divergence within the ingroup of 0–4%.

Molecular MP analysis of tribe Gloxinieae. — Maximum parsimony analysis of the ITS Gloxinieae dataset resulted in 24,000 most-parsimonious trees (length = 1057 steps, CI = 0.486, RI = 0.666, RC = 0.324). Figure 3 is the strict consensus of these trees. The

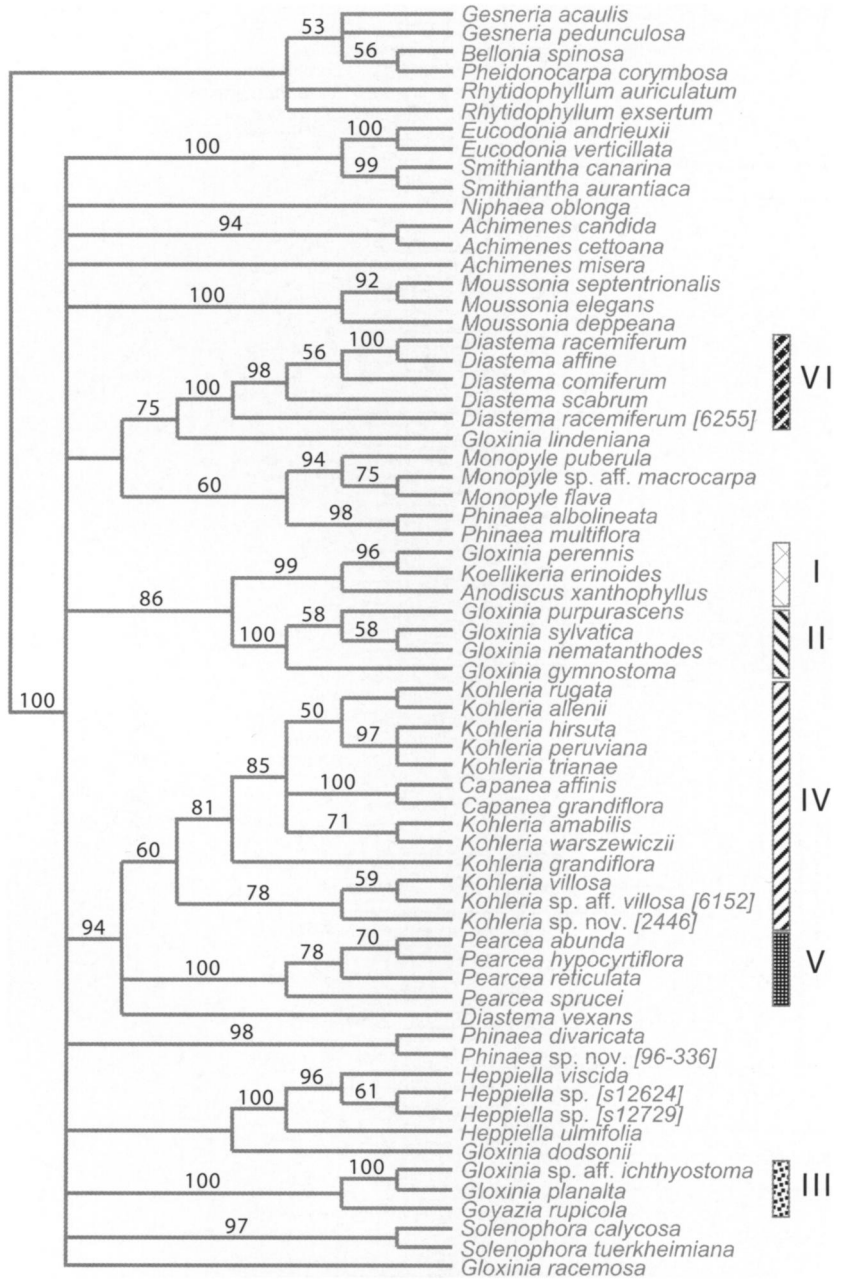

Fig. 6. Analysis of relationships within Gloxinieae using the nrDNA ITS and cpDNA *trnL-F* datasets. Strict (MP) consensus tree of 1097 most-parsimonious trees of 1258 steps. Numbers above branches are bootstrap percentages where branch support is equal to or greater than 50%. Major well-supported clades from the combined analyses and discussed in the text are labeled: I, *Gloxinia*-type clade; II, *Seemannia* clade; III, Brazil clade; IV, *Kohleria* clade; V, *Pearcea* clade; VI, *Diastema* clade.

ITS analysis supports relationships among many groups of closely related species, particularly relationships among species within genera, but relationships among these genera are not supported in most cases. Most of the branches grouping genera collapse in the strict consensus resulting in a large polytomy at the base of tribe Gloxinieae.

The *trnL-F* Gloxinieae dataset maximum parsimony analysis resulted in 128 most-parsimonious trees (length = 171 steps, CI = 0.871, RI = 0.915, RC = 0.797). Figure 4 is the strict consensus of these trees. As with the ITS analysis, the *trnL-F* analysis resulted in a minimally resolved strict consensus, although resolution is in different portions of the trees resulting from the different gene regions (Figs. 3 & 4). Most relationships are not strongly supported, but as with the ITS results, relation-

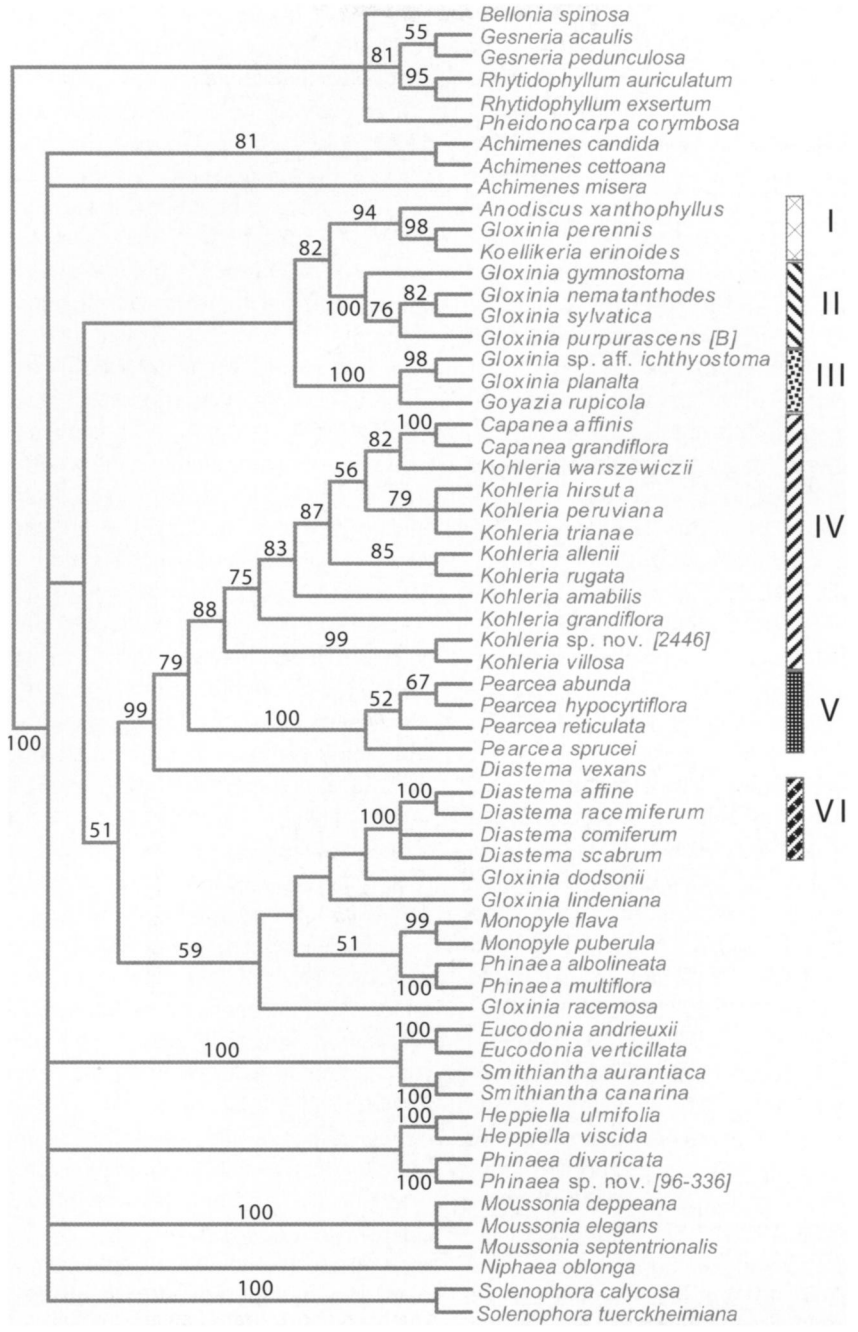

Fig. 7. Analysis of relationships within Gloxinieae using the nrDNA ITS, cpDNA *trnL-F*, and morphology datasets. Strict (MP) consensus tree of 126 most-parsimonious trees of 1586 steps. Numbers above branches are bootstrap percentages where branch support is equal to or greater than 50%. Major well-supported clades from the combined analyses and discussed in the text are labeled: I, *Gloxinia*-type clade; II, *Seemannia* clade; III, Brazil clade; IV; *Kohleria* clade, V, *Pearcea* clade; VI, *Diastema* clade.

ships grouping species within genera are often well supported.

Morphological MP analysis of tribe Gloxinieae. — Maximum parsimony analyses of the Gloxinieae morphology datasets resulted in: all characters equal weight, 6138 most-parsimonious trees (length = 416 steps, CI = 0.200, RI = 0.680, RC = 0.136); flower and inflorescence characters half weight, 4500 most-parsimonious trees (324.5 steps, CI = 0.210, RI = 0.686, RC = 0.144); fruit and seed characters half weight, 47 most-parsimonious trees (388 steps, CI = 0.200, RI = 0.677, RC = 0.135); leaf and stem characters half weight, 3384

most-parsimonious trees (length = 375.5 steps, CI = 0.196, RI = 0.687, RC = 0.134); indument characters half weight, 2341 most-parsimonious trees (377.5 steps, CI = 0.207, RI = 0.689, RC = 0.142).

The most resolved strict consensus tree resulted from the MP analysis with fruit and seed characters downweighted. This strict consensus is presented in Fig. 5. It should be noted that this topology is being presented because it was the most resolved and allows us to map bootstrap values from all of the different weighting analyses on a single tree, not because we necessarily believe this topology to be the most accurate. The bootstrap branch support values of all analyses are listed in Appendix 4 in comparison to this topology. There is low support for most branches under all of the weighting schemes (Fig. 5; Appendix 4).

Combined analysis of tribe Gloxinieae. — Three partitions were explored here with the partition homogeneity test: ITS/*trnL-F*, ITS/morphology, and *trnL-F*/morphology. The partition homogeneity test found a significant difference between the ITS/*trnL-F* partition and random partitioning (P = 0.0274). The partition homogeneity test also found a significant difference between the ITS and morphological datasets (P = 0.00005) and the *trnL-F*/morphology partition (P = 0.0127). Maximum parsimony analysis of the combined ITS/*trnL-F* Gloxinieae dataset resulted in 1097 most-parsimonious trees (length = 1258 steps, CI = 0.529, RI = 0.688, RC = 0.364). Figure 6 is the strict consensus of these trees. The combined ITS/*trnL-F*/morphology (all characters equal weight) Gloxinieae dataset maximum parsimony analysis resulted in 126 most-parsimonious trees (length = 1586 steps, CI = 0.461, RI = 0.648, RC = 0.299). Figure 7 is the strict consensus of these trees.

Conflict between datasets. — Generally, the ITS and cpDNA individual analyses are congruent, with some slight differences among poorly supported nodes (Figs. 3 and 4). Two sets of moderately supported nodes are in conflict. In the ITS MP analysis, *Achimenes candida* and *A. cettoana* are strongly supported as sister taxa (bs = 94%), while the *trnL-F* MP analysis supports *A. cettoana* and *A. misera* as sister taxa (bs = 90%). Similarly, the ITS and *trnL-F* analyses suggest slightly different placements of *Pearcea hypocyrtiflora* (Figs. 3 and 4). The ITS MP analysis supports *P. abunda*, *P. reticulata*, and *P. sprucei* as a clade (bs = 77%) that is sister to *P. hypocyrtiflora* (bs = 100%). Alternatively, the *trnL-F* MP analysis suggests that *P. abunda*, *P. hypocyrtiflora*, and *P. reticulata* form a clade (bs = 88%), with *P. sprucei* sister to this clade (bs = 69%).

The morphology MP analyses suggest a variety of different topologies depending on their weighting scheme, although the majority of the nodes are poorly supported in these analyses, regardless of character weights. While there are significant partition homogeneity test values for the morphology dataset in comparison with both the ITS and *trnL-F* datasets, there are very few differences in the topologies that cross well supported nodes (bs ≥ 70%). The few that do cross these nodes include the placement of *Bellonia* in the Gloxinieae clade by morphology rather than the Gesnerieae clade as found with ITS and *trnL-F*; the exact relationships within *Pearcea* (incongruent with both ITS and *trnL-F*); and the relationships within *Moussonia* (incongruent with ITS; Figs. 3–5).

There is as yet no consensus as to when datasets should be combined (reviewed in de Queiroz & al., 1995). While the partition homogeneity test provides a test of congruence among datasets, it is not clear how the test is affected by differences in gene history vs. homoplasy (Miller & al., 1999). Additionally, many authors consider simultaneous analysis of all data to be the most effective way to study evolutionary descent (Thornton & DeSalle, 2000, and references therein). In this study, the incongruences between the datasets do not generally involve well-supported branches, with the exception of the two cases noted above. In fact, the relationships that this study is most interested in addressing (generic relationships) are generally not in strong conflict in the separate analyses. Therefore, the molecular datasets were combined (Fig. 6) and the two molecular datasets were combined with the morphological dataset (Fig. 7) to optimize the resolving power of the data.

The combined molecular data parsimony analysis trees are a hybrid between the ITS and *trnL-F* topologies (Figs. 3, 4). Strongly supported nodes based on only one dataset are generally present and strongly supported in the combined analysis (e.g., the "*Seemannia*" *Gloxinia* clade; Clades I–VI, Figs. 3, 4, 6). There are some cases where clades strongly supported by one dataset have less support in the combined analysis than in either of the separate analyses, but these are infrequent (e.g., relationships within *Pearcea*; Figs. 3, 4, 6). The addition of morphology to the combined molecular datasets provides a very similar topology, with increased bootstrap support at well supported nodes in the molecular analyses. Generally, the combined analyses represent the strongly supported nodes of each of the individual analyses.

Some might argue that given the apparent homoplasy of the morphological characters, these data should not be included in the phylogenetic analyses. It should be noted, however, that if the morphological data provided no phylogenetic structure, or only structure in conflict with the molecular datasets, then we would expect that the inclusion of the morphological dataset would result in a reduction in resolution of the strict consensus and a reduction in bootstrap values across the phylogeny. In actuality, while there is clearly homoplasy in the mor-

phological characters, these characters do increase resolution and branch confidence over the molecular data alone. The ITS/*trnL-F* strict consensus has 73.44% of the possible nodes resolved (47 of 64 nodes) while the molecular data combined with morphology has 81.36% of the possible nodes resolved (48 of 59 nodes). Branch support generally increases with the addition of the morphological dataset with the percentage of nodes with a bs ≥ 70% increasing from 51.56% to 57.63% and the percentage of nodes with a bs ≥ 95% increasing from 32.81% to 35.59% (Figs. 6–7). This pattern suggests that there is phylogenetic signal congruent with the molecular data underlying the homoplastic variation present in the morphological characters. Because of this we consider the inclusion of the morphological dataset appropriate and the three dataset consensus tree to be our best current estimate of relationships in Gloxinieae.

DISCUSSION

Generic membership of Gesnerieae and Gloxinieae. — Tribal organization in Gesnerioideae has varied significantly over the last 150 years (Wiehler, 1983). Much of this circumscriptional variation has revolved around the inclusion and exclusion of certain genera from the core members of tribes Episcieae, Gesnerieae, and Gloxinieae. Molecular phylogenetic analyses are now providing concrete evidence for tribal circumscription where morphological characters, and some molecular datasets, have failed in the past (Wiehler, 1983; Smith, 1996, 2000, 2001; Smith & al., 1997; Smith & Atkinson, 1998). It appears clear that *Sinningia* and its related genera should be classified as a tribe separate from Gloxinieae, where they have been included by Wiehler (1983) and Burtt & Wiehler (1995), as they are not closely related to the core Gloxinieae clade (Fig. 2; Smith & al., 1997; Zimmer & al., 2002; Perret & al., 2003). In accordance with these results, the tribe Sinningieae has been recently segregated from tribe Gloxinieae (Weber, 2004). Additionally, there are several other genera whose placement has been problematic, namely *Bellonia*, *Lembocarpus*, and *Pheidonocarpa*.

From the results documented in Fig. 2, it is clear that *Bellonia* and *Pheidonocarpa* are members of Gesnerieae. Skog (1976) originally described *Pheidonocarpa* as a member of Gesnerieae, but given that this monotypic genus is missing one of the primary synapomorphies for this tribe (alternate leaves), its tribal placement has varied (Skog, 1976). Prior to the creation of the genus *Pheidonocarpa*, the species *P. corymbosa* had been classified in the genera *Gesneria*, *Pentaraphia*, and *Heppiella*. *Bellonia*, on the other hand, has not been previously considered to be a member of Gesnerieae, being placed either in tribe Bellonieae (Fritsch, 1893–1894) or in tribe Gloxinieae (Wiehler, 1983). Morphologically, *Bellonia* is difficult to place due to its woody stems, opposite leaves, lack of underground storage structures, and rotate corollas (Xu & Skog, 1990). This combination of characters is rare in Gesnerioideae, and has led to confusion on the proper placement of the genus. Fritsch (1893–1894) circumscribed the tribe Bellonieae to include all of the species that have rotate corollas (*Bellonia*, *Niphaea*, and *Phinaea*). In all analyses presented here based on molecular, morphological, and combined analyses, this grouping of all of the rotate-corolla species in one lineage is refuted. The morphological analyses do place *Bellonia* and *Niphaea* together, but this relationship is not supported in the molecular analyses nor in the combined data analyses (Figs. 3–7). While *Bellonia* does not possess two key synapomorphies for the rest of Gesnerieae, it does share geographic distribution with the rest of the tribe as it is restricted to the Caribbean region, as are all of the other genera of Gesnerieae (Xu & Skog, 1990). Relationships in Gesnerieae need to be further explored, particularly how the genera *Bellonia* and *Pheidonocarpa* relate to *Gesneria* and *Rhytidophyllum*, as well as the patterns of morphological diversification in the tribe.

Lembocarpus has been similarly difficult to place since its description in 1958 (Leeuwenberg, 1958). It has been alternatively associated with members of tribe Episcieae (Leeuwenberg, 1958; Beaufort-Murphy, 1983; Boggan, 1991) and tribe Gloxinieae (Wiehler, 1983; Burtt & Wiehler, 1995; Smith, 2001). The chromosome number, which would help associate this genus with one tribe or the other, remains unknown. The primary rationale for placing *Lembocarpus* in Gloxinieae (sensu Wiehler, 1983) is the presence of a tuber, suggesting a possible relationship to *Sinningia* and relatives (Wiehler, 1983). Others have suggested it holds affinity with Episcieae based on geographic distribution and morphological characters such as seed shape (Beaufort-Murphy, 1983; Boggan, 1991). The mophological characteristics of *Lembocarpus* also are discussed in detail by Feuillet & Skog (2003) in comparison with the newly described genus *Cremersia*. *Lembocarpus* has been included in one previous molecular phylogenetic analysis (Smith, 2001), which placed it in tribe Gloxinieae, although branch support in these analyses was low and there are questions of accuracy of these sequences (Zimmer & al., 2002). It is interesting that two of the results from Smith (2001) are incongruent with the results presented here: the polyphyly of *Capanea* and the placement of *Lembocarpus* in Gloxinieae. *Lembocarpus* was placed in the study by Smith (2001) as sister to *Capanea affinis*, and *Capanea grandiflora* was placed with *Chrysothemis*, *Nautilocalyx*, and *Paradrymonia* in Episcieae. That placement of these

two species is almost exactly opposite from this study, suggesting that these samples may have been transposed. It appears clear now that *Lembocarpus* belongs in Episcieae (Fig. 2) and is possibly closely related to the genera *Chrysothemis*, *Nautilocalyx*, and *Paradrymonia*. These genera are quite diverse in northeastern South America, the biogeographic region to which *Lembocarpus* is restricted (Wiehler, 1983), and species of *Chrysothemis* and *Nautilocalyx* are known to possess tubers, in congruence with this suggested relationship.

Despite the falsification of Wiehler's placement of *Bellonia*, *Lembocarpus*, and *Sinningia* and relatives in Gloxinieae, much of his reorganization of Gloxinieae from previous classifications (Bentham, 1876; Fritsch, 1893–1894; Ivanina, 1965; for overview see Wiehler, 1983) is supported by these analyses. Particularly, the expanded circumscription of tribe Gloxinieae to include Kohlerieae, Solenophoreae, and part of Bellonieae (i.e., *Niphaea* and *Phinaea*) is strongly supported (Figs. 2–4, 6–7).

Morphological cladistic analysis and the classifications of Fritsch (1893–1894). — The morphological cladistic strict consensus presented in Fig. 5 provides an interesting topology to compare with different generic classification schemes, from the early classifications of Fritsch (1893–1894) to the reorganizations of these genera largely by Wiehler (see Wiehler, 1983, for overview). Particularly interesting is the presence of clades in the morphological analyses (Fig. 5) that are very similar to parts of Fritsch's organization of *Achimenes* s.l. and *Kohleria* s.l. Fritsch had broad views of these two genera: what Wiehler considered parts of *Achimenes*, *Eucodonia*, *Gloxinia*, and *Goyazia*, Fritsch included in *Achimenes*, and what Wiehler considered parts of *Kohleria*, *Moussonia*, and *Parakohleria*, Fritsch included in *Kohleria*. The consensus topology shown (Fig. 5) reflects morphological signal that Fritsch likely used in his classification. A clade is present in the morphological analyses that is similar to Fritsch's concept of *Achimenes*, including *Achimenes* s.s., *Eucodonia*, parts of *Gloxinia*, and *Goyazia*, although the clade also includes *Koellikeria* and *Smithiantha* (Fig. 5). The morphological cladistic analyses reflect a clade with *Pearcea/ Parakohleria* nested within *Kohleria*, although *Moussonia* is placed separate from *Kohleria* s.l. While we now expect these classifications of Fritsch to not reflect evolutionary relationships (given our other results), the association of historical generic concepts with morphological cladistic analyses is interesting, and likely reflects Fritsch's careful observations of morphological variation.

Generic boundaries in Gloxinieae. — The results of these analyses support the monophyly of several genera including *Capanea*, *Eucodonia*, *Heppiella*, *Monopyle*, *Moussonia*, *Pearcea*, *Smithiantha*, and *Solenophora* (Figs. 3, 4, 6, 7). Previous authors have separated some species here considered *Pearcea* (following Kvist & Skog, 1996) into the genus *Parakohleria* (Wiehler, 1978, 1983). All of the samples from these two possible genera group together quite strongly (Figs. 3, 4, 6, 7). The type species of *Pearcea*, *P. hypocyrtiflora*, is nested within the species sometimes considered part of *Parakohleria* in the *trnL-F* and combined analyses (Figs. 4, 6, 7). As the recognition of *Parakohleria* would result in the genus being paraphyletic, we suggest that all of these species are best dealt with as a single genus. This has been previously suggested based on morphology by Kvist & Skog (1996), who noted that the morphological characters used to distinguish *Parakohleria* from *Pearcea* (*Pearcea* having 1–2 flowered inflorescences, bilobed stigmas, and an inability to hybridize with *Parakohleria*) did not hold up with the addition of new *Pearcea* species described by Kvist & Skog (1996). Particularly they note that the stigmas of *Pearcea* s.s. are only weakly bilobed and are more similar to the usually capitate stigmas of *Parakohleria* than the bilobed stigmas of *Kohleria*. While the recognition of *Parakohleria* separate from *Pearcea* is not supported, the movement of several species out of *Kohleria* to *Parakohleria/Pearcea* by Wiehler (1978) is supported by these analyses as all of the *Pearcea/Parakohleria* species are well supported as a clade separate from, although sister to, the *Kohleria* clade (Clades IV & V, Figs. 3, 4, 6, 7).

The recognition of *Moussonia* as a genus in its own right (Regel, 1847, 1848; Wiehler, 1975a, 1983), rather than a part of *Kohleria* (Bentham, 1876; Fritsch, 1893–1894) is here supported (as in Zimmer & al., 2002) with *Moussonia* clearly a separate clade from *Kohleria* (Figs. 6, 7). For a detailed history of the classification of *Moussonia*, see Wiehler (1975a). It is interesting to note that although Fritsch's union of *Moussonia* with *Kohleria* was based on morphology, not even our morphological analyses support a close relationship between these two genera. While *Moussonia* is clearly not closely related to *Kohleria*, its phylogenetic proximity to any other genus is not clear in these analyses (Figs. 6, 7). Further studies will be necessary to determine what relationship *Moussonia* has with the other genera of Gloxinieae.

Kohleria generally forms a clade with *Capanea* nested within it (Figs. 3, 4, 6, 7). At least one node with a bootstrap > 70% places *Capanea* within *Kohleria* in the ITS, ITS + *trnL-F* and ITS + *trnL-F* + morphology analyses (Figs. 3, 6, 7). As *Capanea* and *Kohleria* share several morphological characteristics and primarily differ by *Capanea* being epiphytic and *Kohleria* terrestrial and *Capanea* having a stomatomorphic stigma and *Kohleria* being bilobed (Kvist & Skog, 1992), it is not surprising

that there is a close phylogenetic affinity. It appears that the specialized habit of *Capanea* has been derived from the more generalized habit of *Kohleria*. The similarity between *Capanea* and *Kohleria* species has been previously noted (Kvist & Skog, 1992). Particularly, Kvist & Skog (1992) noted how the tubular to campanulate corollas, pedunculate axillary inflorescences, and rotundate calyx lobes of *Capanea affinis* and *Kohleria warszewiczii* were "strikingly similar". Interestingly, *K. warszewiczii* is placed sister to the *Capanea* clade in the three dataset analysis (Fig. 7). The taxonomic implications of a *Capanea* clade nested within *Kohleria* are being addressed elsewhere (Roalson & al., in prep.).

In the most recent revision of *Kohleria*, Kvist & Skog (1992) recognized 25 taxa in 17 species. We have here sampled nine of the 17 species recognized in the revision and can make some general comments about relationships in the genus, beyond its paraphyletic relationship with *Capanea*. It has been suggested that interspecific hybridization may frequently occur in *Kohleria*, with reported hybrids among several species common (Kvist & Skog, 1992). It has also been noted that many of the *Kohleria* species grow sympatrically with other *Kohleria* species (Kvist & Skog, 1992). The two molecular datasets presented here do not give any evidence for hybridization among these collections, although the relationships within *Kohleria* based on the *trnL-F* sequences are nearly completely unresolved (Fig. 4). The trees from combined analyses are relatively well-resolved and supported, and suggest that *K. villosa* and relatives may be sister to the rest of the genus, at least as sampled here. Additionally, there are several supported species pairs or triplets, including a *K. hirsuta/K. peruviana/K. trianae* clade, a *K. allenii/K. rugata* clade, and possibly a *K. amabilis/K. warszewiczii* clade, although this third clade is not present in the combined molecular/morphology analysis (Fig. 7). Some species previously suggested to be similar and likely to be closely related are supported as such here, including *K. hirsuta* and *K. peruviana*, but other species sometimes suggested to be closely related or morphologically similar do not group together in these analyses, particularly *K. amabilis*, *K. rugata*, and *K. villosa* (Kvist & Skog, 1992; Figs. 6, 7).

The phylogenetic relationships within *Achimenes* have been dealt with elsewhere (Roalson & al., 2003). The monophyly of *Achimenes*, and its relationship to the genera *Niphaea* and *Solenophora*, is unclear at this time. It is possible that *Solenophora* and/or *Niphaea* are nested within the current limits of *Achimenes*, but due to the lack of support for these branches and their collapse in the strict consensus trees presented here, further studies will be necessary to determine these relationships conclusively (Figs. 3, 4, 6, 7). Wiehler's (1976) separation of *Eucodonia* from *Achimenes* is here supported with *Eucodonia* clearly more closely related to *Smithiantha* than *Achimenes* (Figs. 3, 4, 6, 7).

Three genera appear to be polyphyletic assemblages: *Diastema*, *Gloxinia*, and *Phinaea*. *Diastema* forms a strongly supported clade with the exclusion of *D. vexans*, which appears to be more closely related to *Pearcea* and *Kohleria* than to the rest of *Diastema* (Figs. 3–4, 6–7). This is strongly supported in the combined analyses. The only character that appears to separate *D. vexans* from the rest of *Diastema* is the inflorescence structure: a reduced axillary, bracteolate, pair-flowered cyme in *D. vexans* vs. solitary axillary flowers arranged in a raceme-like terminal flowering stem in the rest of *Diastema*. Potential morphological characters supporting these relationships will be further explored elsewhere (Roalson & al., unpubl.).

The three or four sampled species of *Phinaea* (depending on the analysis) consistently form two separate clades, with the placement of each of these clades weakly supported in all analyses, but consistently not grouping together (Figs. 3, 4, 6, 7). These two separate clades appear to be supported by a series of morphological characters, including fruit type and orientation and nectary type, and these characteristics will be further explored elsewhere (Roalson & al., unpubl.).

Gloxinia is the most problematic genus in these analyses and has also undergone major reorganization in the last 30 years (Wiehler, 1972, 1975b, 1976, 1983). *Gloxinia* was considered by Fritsch (1893–1894) to be a monotypic genus, including only *G. perennis*. The most recent revisions have moved species from the genera *Achimenes*, *Kohleria*, *Monopyle*, and *Seemannia* (Wiehler, 1976, 1983) into *Gloxinia*. Wiehler's (1976) enlarged circumscription of *Gloxinia* has no clear morphological basis; it appears to be based primarily on interspecific hybridization, although these hybrids were almost entirely between *G. perennis* and species of the *Seemannia* group (*G. gymnostoma*, *G. nematanthodes*, *G. purpurascens*, *G. sylvatica*; Clade II, Figs. 3, 4, 6, 7). Eleven of the approximately 15 currently recognized species of *Gloxinia* are here included in the phylogenetic analyses. *Gloxinia* as currently circumscribed is polyphyletic, with species in seven different clades (Figs. 2–4, 6, 7). This result is congruent with some morphological and biogeographic characters, as well as previous views of generic boundaries. Particularly, all of the species sampled that were previously included in the genus *Seemannia* are strongly supported as a monophyletic group in combined data analyses (Figs. 6, 7). These species appear to share several suites of morphological characteristics not found in the rest of *Gloxinia* or any other genus of Gloxinieae (Roalson & al., unpubl.).

The type species of *Gloxinia*, *G. perennis*, is clearly closely related to the monotypic genera *Anodiscus* and

Koellikeria (Figs. 3, 4, 6, 7). All three species share a similar inflorescence type (a raceme-like flowering stem with flowers solitary in the axils of strongly reduced leaves) and are strongly supported as a clade by combined analyses (Figs. 6, 7; bs = 99% and 94%, respectively).

Two *Gloxinia* species, *G.* sp. aff. *ichthyostoma* and *G. planalta*, group strongly with *Goyazia rupicola* (Figs. 6, 7). All of the species in this clade are endemic to Brazil, outside of the range of typical *Gloxinia* species. The placement of *Goyazia* has historically been considered to be unclear (see Wiehler, 1976), but this genus has for the last 25 years been considered a member of Gloxinieae sensu Wiehler (Wiehler, 1976, 1983; Smith, 2001). Our data clearly support Wiehler's placement of *Goyazia* in Gloxinieae. While species boundaries in this group of species are problematic (J. K. Boggan, pers. obs.; A. Chautems, pers. comm.), it seems clear that at least some of the species of Gloxinieae found in central Brazil form a clade (excluding *Gloxinia sarmentiana*; see Zimmer & al., 2002).

Three other species of *Gloxinia*, *G. dodsonii*, *G. lindeniana*, and *G. racemosa*, are included here and appear to form three separate lineages, although in some analyses *G. dodsonii* and *G. lindeniana* are placed as relatively closely related (Figs. 4 & 7). *Gloxinia dodsonii*, *G. lindeniana*, and *G. racemosa* only share morphological similarity to *Diastema*, *Monopyle*, and *Phinaea* p.p. (all sometimes forming a clade; Figs. 3, 4, 6, 7) in regards to having a fleshy fruit with a splitting hypanthium. Otherwise, this grouping is morphologically quite diverse. Morphological characters associated with these relationships will be further discussed elsewhere (Roalson & al., in prep.).

Conclusions. — While phylogenetic relationships among the genera of Gloxinieae are not completely resolved, it is clear that the circumscription of several genera, particularly *Gloxinia* and *Phinaea*, require revision. *Diastema* is a morphologically coherent genus and the unexpected placement of *D. vexans* in a separate lineage from the rest of the genus merits further study. The expanded concept of Gloxinieae sensu Wiehler (1983) is largely supported with the exception of Wiehler's inclusion of *Bellonia*, *Lembocarpus*, and tribe Sinningieae. Several major clades in Gloxinieae are supported, including an *Anodiscus/Gloxinia* s.s./*Koellikeria* clade, a *Seemannia*-type *Gloxinia* clade, a central Brazil *Gloxinia/Goyazia* clade, a *Capanea/Diastema vexans/Kohleria/Pearcea* clade, and a *Eucodonia/Smithiantha* clade. Wiehler's segregation of *Eucodonia* from *Achimenes* and *Moussonia* from *Kohleria* are justified, but his segregation of Para*kohleria* from *Pearcea* is not. The genera *Heppiella*, *Moussonia*, and *Phinaea* p.p. appear to be either isolated in the tribe without close relatives, or have as yet unresolved relationships to the other genera of tribe Gloxinieae. The relationships need to be further explored through the addition of taxa and new datasets to help resolve the internal nodes grouping major clades in the tribe. The evolution of morphological characteristics in this tribe will be explored in more detail in future papers.

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Appendix 1. Samples taken from live material growing at the U.S. Botany Research Greenhouses (USBRG) are designated by their accession number and a voucher number, when present. All voucher specimens are deposited at US unless otherwise noted. Herbarium acronyms are as follows: MSB = Institut für systematische Botanik, München, Germany; US = United States National Herbarium, Smithsonian Institution, Washington, D.C., U.S.A.; and UVAL = Universidad del Valle de Guatemala, Guatemala City, Guatemala. Localities of original collections are listed, where known. Material derived from cultivated collections with unknown original localities are listed as "unknown" followed by their known geographic range. Notes in brackets refer to collection designations when multiple collections of the same taxon are referred to in the text or figures.

Species, Voucher and locality, ITS GenBank Accession, *trnL-F* GenBank Accession

Achimenes candida Lindl., USBRG 96-150, *Skog 7840*, unknown (Central America), AY047065, AY047124; ***A. cettoana*** H.E.Moore, USBRG 94-235, *Skog 7991*, unknown (Mexico), AY047066, AY047125; ***A. misera*** Lindl., USBRG 94-475, *Skog 7903*, unknown (Central America), AY047067, AY047126; ***Aeschynanthus hildebrandii*** Hemsl., USBRG 94-254, *Skog 7777*, unknown (Thailand), AY047040, AY047099; ***Alloplectus bolivianus*** (Britton) Wiehler, USBRG95-140, *Mendoza-T. et al. 506*, Ecuador, AY047097, AY047156; ***Anodiscus xanthophyllus*** (Poepp.) Mansf., USBRG 99-041, *Skog 8140*; unknown (Ecuador and Peru), AY047074, AY047133; ***Bellonia spinosa*** Swartz, *M. J. Evans s.n.* (fresh material from M. J. Evans, not vouchered), unknown (Cuba and Hispaniola), AY702350, AY702394; ***Besleria labiosa*** Hanst., USBRG 85-093, *Skog 7631*, Venezuela, AY047041, AY047100; ***Capanea affinis*** Fritsch, *J. L. Clark 5790*, Ecuador, AY702351, AY702395; ***C. grandiflora*** (Kunth) Decne. ex Planch., *J. L. Clark 6041*, Ecuador, AY702352, AY702396; ***Chrysothemis pulchella*** (Donn ex Sims) Decne., USBRG 82-092, *Skog 5714*, Venezuela, AY047085, AY047144; ***Codonanthe carnosa*** (Gardner) Hanst., USBRG 94-471, *Skog 8054*, unknown (Brazil), AY047088, AY047147; ***Columnea spathulata*** Mansf., USBRG 95-153, *Skog 7820*, Ecuador, AY047092, AY047151; ***Corytoplectus cutucuensis*** Wiehler, USBRG 94-021, *Dunn 9405017*, Ecuador, AY047094, AY047153; ***Diastema affine*** Fritsch, *J. L. Clark 6034*, Ecuador, AY702353, AY702397; ***D. comiferum*** (DC.) Benth. ex Walp., USBRG 01-259, *Skog 8259*, Peru, AY702354, AY702398; ***D. racemiferum*** Benth. (1), USBRG 96-258, *Skog 8052*, Ecuador, AY047069, AY047128; ***D. racemiferum*** Benth. (2), *J. L. Clark 6255*, Ecuador, AY702355, AY702399; ***D. scabrum*** (Poepp.) Benth. ex Walp., *J. L. Clark 5631*, Ecuador, AY702356, AY702400; ***D. vexans*** H.E.Moore, USBRG 02-153, *Skog 8260*; unknown (Colombia), AY702357, AY702401; ***Drymonia serrulata*** (Jacq.) Mart., USBRG 94-109, *Skog 7876*, unknown (widespread in Central and South America), AY047093, AY047152; ***Episcia punctata*** (Lindl.) Hanst., USBRG 77-103, *Skog 5349*, Mexico, AY047090, AY047149; ***Eucodonia andrieuxii*** (DC.) Wiehler, USBRG 92-022, *Skog 7620*, unknown (Mexico), AY047060, AY047119; ***E. verticillata*** (M.Martens & Galeotti) Wiehler, USBRG 86-097, *Skog 7714*, unknown (Mexico), AY047061, AY047120; ***Gasteranthus quitensis*** Benth., USBRG 95-152, *Mendoza-T. et al. 525*, Ecuador, AY047042, AY047101; ***Gesneria acaulis*** L., USBRG 97-116, *MBG 140-69* (Montreal Botanic Garden living collection voucher number), Jamaica, AY047045, AY047104; ***G. citrina*** Urb., USBRG 97-206, not vouchered; Puerto Rico, AY047054, AY047113; ***G. cuneifolia*** (DC.) Fritsch, USBRG 98-006; not vouchered; Puerto Rico, AY047047, AY047106; ***G. pedunculosa*** (DC.) Fritsch, USBRG 97-204, *Skog 8084*, Puerto Rico, AY047052, AY047111; ***Gloxinia dodsonii*** Wiehler, USBRG 02-109, *Mendoza-T. et al. 554*, Ecuador, AY702358, AY702402; ***G. gymnostoma*** Griseb., USBRG 02-108, not vouchered, unknown (Argentina or Bolivia), AY702359, AY702403; ***G.* sp. aff. *ichthyostoma*** Gardner, USBRG 99-002, *Skog 8219*; Brazil, AY702360, AY702404; ***G. lindeniana*** (Regel) Fritsch, USBRG 95-083, not vouchered, taxon known only from cultivation, precise origin unknown, but South American, likely from Andes, AY702361, AY702405; ***G. nematanthodes*** (Kuntze) Wiehler, USBRG 02-107, not vouchered, unknown (Argentina or Bolivia), AY702362, AY702406; ***G. perennis*** (L.) Fritsch, USBRG 95-081, *Skog 7723*, Ecuador, AY047071, AY047130; ***G. planalta*** Wiehler, *Fonseca 766*, Brazil, AY702363, -, ***G. purpurascens*** (Rusby) Wiehler [B], USBRG 98-079; *Skog 7839*; Bolivia, AY047072, AY047131; ***G. racemosa*** (Benth.) Wiehler, USBRG 02-155; *Skog 8258*; Colombia, AY702364, AY702407; ***G. sarmentiana*** Gardner ex Hook., USBRG 00-148; *Skog 8262*; Brazil, AY047079, AY047138; ***G. sylvatica*** (Kunth) Wiehler, USBRG 96-002, *Skog 7760*, Bolivia, AY702365, AY702408; ***Goyazia rupicola*** Taubert, *Irwin 19257*, Brazil, AY702366, AY702409; ***Heppiella* sp.** [s12624], *R. Dunn 9604081*, USBRG 96-323, *Stewart* (RES) *12624* (all living collection numbers), Ecuador, AY702367, AY702410; ***H.* sp.** [12729], *R. Dunn 9604233*, *Stewart* (RES) *12729* (both living collection numbers), Ecuador, AY702368, AY702411; ***H. ulmifolia*** (Kunth) Hanst., USBRG 01-260, *J. L. Clark 2418*; Ecuador, AY702369, AY702412; ***H. viscida*** (Lindl. & Paxt.) Fritsch, 99-2256; not vouchered; unknown (Venezuela), AY702370, AY702413; ***Koellikeria erinoides*** (DC.) Mansf., USBRG 77-232; *Skog 7596*; unknown (widespread in Central and South America), AY047073, AY047132; ***Kohleria allenii*** Standl. & L.O.Wms., USBRG 98-109; *Skog 8056*; Costa Rica, AY702371, AY702414;

Appendix 1 (continued).

K. amabilis (Planch. & Linden) Fritsch, USBRG 99-102, *Skog 8042*, Colombia, AY702372, AY702415; ***K. grandiflora*** L.P.Kvist & L.E.Skog, USBRG 98-259, *Skog 8188*, Ecuador, AY702373, AY702416; ***K. hirsuta*** (Kunth) Regel, USBRG 96-163, *Skog 7782*, Ecuador, AY702374, AY702417; ***K. peruviana*** Fritsch, USBRG 94-462, *Skog 8145*, unknown (Peru), AY702375, AY702418; ***K. rugata*** (Scheidw.) L.P.Kvist & L.E.Skog, USBRG 95-010, *Skog 7766*, Mexico, AY047075, AY047134; ***K.* sp. nov.** [2446], *J. L. Clark 2446*, Ecuador, AY702376, AY702419; ***K. trianae*** (Regel) Hanst., USBRG 94-399, not vouchered, unknown (Colombia and Ecuador), AY702377, AY702420; ***K.* sp. aff. *villosa*** (Fritsch) Wiehler [6152], *J. L. Clark 6152* (Ecuador), AY702378, AY702421; ***K. villosa*** (Fritsch) Wiehler [8041], USBRG 98-031, *Skog 8041*, Ecuador, AY047076, AY047135; ***K. warszewiczii*** (Regel) Hanst., USBRG 94-541, not vouchered, unknown (Colombia), AY702379, AY702422; ***Lembocarpus amoenus*** Leeuwenb., USBRG 02-110; *J. F. Smith 4125*, French Guiana, AY702380, AY702423; ***Monopyle flava*** L.E.Skog, USBRG 96-149, *Skog 8055*, Ecuador, AY702381, AY702424; ***M.* sp. aff. *macrocarpa*** Benth., *J. L. Clark 5638*, Ecuador, AY702382, AY702425; ***M. puberula*** C.V.Morton (previously misidentified as *M. macrocarpa* Benth.), USBRG 95-060, *Skog 7880*, Costa Rica, AY047070, AY047129; ***Moussonia deppeana*** (Schlechtend. & Cham.) Hanst., USBRG 01-254, *Skog 8231*, unknown (Central America), AY702383, AY702426; ***M. elegans*** Decne., USBRG 01-253, not vouchered, Costa Rica, AY702384, AY702427; ***M. septentrionalis*** (Denham) Wiehler, USBRG 99-001, *Skog 8047*, Mexico, AY047068, AY047127, ***Napeanthus jelskyi*** Fritsch, USBRG 94-511, *Skog 7697*, French Guiana, AY047044, AY047103; ***Nautilocalyx melittifolius*** (L.) Wiehler, USBRG 98-256, *Skog 7852*, unknown (Caribbean), AY047086, AY047145; ***Nematanthus strigillosus*** (Mart.) H.E.Moore, USBRG 94-217, *Skog 7751*, Brazil, AY047089, AY047148; ***Neomortonia rosea*** Wiehler, USBRG 96-103, *Skog 8113*, unknown (Costa Rica to Peru), AY047095, AY047154; ***Niphaea oblonga*** Lindl., USBRG 78-354, *Skog 5336*, unknown (Mexico or Guatemala), AY047064, AY047123; ***Paliavana prasinata*** (Ker Gawl.) Fritsch, USBRG 78-360, *Skog 5399*, unknown (Brazil), AY047081, AY047140; ***Paradrymonia binata*** Wiehler, USBRG 96-092 (clone of type collection), Gesneriad Research Foundation 9071, not vouchered, Ecuador, AY047087, AY047146; ***Pearcea abunda*** (Wiehler) L.P.Kvist & L.E.Skog, USBRG 87-032, *Skog 8019*, Ecuador, AY047077, AY047136; ***P. hypocyrtiflora*** (Hook.f.) Regel, USBRG 02-106, *Skog 8270*, unknown (Ecuador), AY702385, AY702428; ***P. reticulata*** (Fritsch) L.P.Kvist & L.E.Skog, *J. L. Clark 5630*, Ecuador, AY702386, AY702429; ***P. sprucei*** (Britton) L.P.Kvist & L.E.Skog, *J. L. Clark 5307*, Ecuador, AY702387, AY702430; ***Pheidonocarpa corymbosa*** (Swartz) L.E.Skog, *Skog 1620*, Jamaica, AY702388, AY702431; ***Phinaea albolineata*** (Hook.) Benth. ex Hemsl., USBRG 02-150, *Skog 8263*, Brazil, AY702389, AY702432; ***P. divaricata*** (Poepp.) Wiehler (previously this sample was referred to as *P. ecuadorana* Wiehler, which we now consider to be synonymous to ***P. divaricata***.), USBRG 98-008, *Skog 8039*, Ecuador, AY047078, AY047137; ***P. multiflora*** C.V. Morton, USBRG 02-151, *Skog 8261*, unknown (Mexico), AY702390, AY702433; ***P.* sp. nov.** [96-336], USBRG 96-336, *Skog 8225*, Ecuador, AY702391, AY702434; ***Reldia minutiflora*** (L.E.Skog) L.P.Kvist & L.E.Skog **var. *minutiflora***, *Hammel 16030*, Ecuador, AY047043, AY047102; ***Rhytidophyllum auriculatum*** Hook., USBRG 97-113, *MBG 937-71* (Montreal Botanic Garden living collection voucher number), unknown (Caribbean), AY047058, AY047117; ***R. exsertum*** Griseb., USBRG 97-114, *Skog 8050*, Cuba, AY047055, AY047114; ***Sinningia cooperi*** (Paxton) Wiehler, USBRG 94-340, *Skog 7808*, unknown (Brazil), AY047082, AY047141; ***S. incarnata*** (Aubl.) Denham, USBRG87-059, *Skog 7784*, Colombia, AY047083, AY047142; ***S. lindleyi*** Schauer, USBRG 97-033, *Skog 7806*, Brazil, AY047084, AY047143; ***Smithiantha aurantiaca*** Wiehler, USBRG 96-392, *Skog 7834*, unknown (Mexico), AY047063, AY047122; ***S. canarina*** Wiehler, USBRG 94-039, *Skog 7684*, Mexico, AY047062, AY047121; ***Solenophora calycosa*** J.D.Sm., *H. Förther et al. 10474* (UVAL, MSB), Guatemala, AY702392, AY702435; ***S. tuerkheimiana*** J.D.Sm., *H. Förther et al. 10433* (UVAL, MSB), Guatemala, AY702393, AY702436; ***Streptocarpus primulifolius*** Gand., USBRG 94-096, *Skog 7868*, unknown (South Africa), AY047039, AY047098; ***Vanhouttea lanata*** Fritsch, USBRG 94-040, *Skog 7712*, Brazil, AY047080, AY047139.

Appendix 2. Morphological characters and character states used in morphological cladistic analyses.

(1.) Chromosome number (base chromosome number in the case of polyploids): (0) $x = 14$, (1) $x = 13$, (2) $x = 12$, (3) $x = 11$, (4) $x = 10$; **(2.) Distribution:** (0) Caribbean, (1) Central America, (2) Andean/Western South American affinity, (3) Southern Brazilian affinity, (4) Guianas; **(3.) Habit:** (0) unlignified weak-stemmed herbs, (1) slightly lignified subshrubs (woody only at base), (2) woody subshrubs or shrubs; **(4.) Location:** (0) not epiphytic (i.e., terrestrial, but also including rupicolous), (1) epiphytic; **(5.) Scaly rhizomes:** (0) absent, (1) present (scales fleshy, usually closely spaced); **(6.) Stringy (whiplike) rhizomes:** (0) absent, (1) present (scales membranous, widely spaced); **(7.) Leaf phyllotaxy:** (0) opposite (i.e., neither ternate nor alternate), (1) consistently or predominantly ternate or whorled, (2) alternate/spiral phyllotaxy; **(8.) Leaf anisophylly:** (0) equal or subequal (i.e., not distinctly anisophyllous), (1) distinctly anisophyllous; **(9.) Leaf petiolation:** (0) petioles not or barely evident, (1) distinct petioles; **(10.) Leaf base equality:** (0) (sub)equal (i.e., not distinctly unequal), (1) distinctly unequal and oblique; **(11.) Leaf veins, number:** (0) 3–5 pairs, (1) 6–8 pairs, (2) 9–14 pairs, (3) 15+ pairs; **(12.) Leaf veins, pattern:** (0) not pericraspedodromous, (1) pericraspedodromous; **(13.) Petiole vasculature:** (0) shallow crescent, (1) deep crescent, (2) a ring (or nearly so); **(14.) Pseudostipules:** (0) absent, (1) present; **(15.) Adaxial surface texture of leaf:** (0) not areolate, (1) areolate; **(16.) Leaf texture:** (0) soft/fleshy/membranous (i.e., not stiff or leathery), (1) stiff/leathery/coriaceous; **(17.) Stomatal domes:** (0) absent, (1) present; **(18.) Stomatal islands:** (0) not aggregated (i.e., randomly scattered), (1) aggregated; **(19.) Subtending leaf internode length:** (0) not becoming reduced, (1) becoming reduced (i.e., more so than vegetative leaves); **(20.) Size of leaf subtending inflorescence:** (0) not small and bract-like (i.e., similar to vegetative leaves), (1) strongly reduced and bract-like; **(21.) Subtending leaf phyllotaxy:** (0) not changing on flowering shoots, (1) changing on flowering shoots; **(22.) Peduncle:** (0) inflorescences epedunculate, (1) inflorescences pedunculate; **(23.) Secondary peduncles:** (0) always absent (i.e., axillary cyme never branching), (1) can be present; **(24.) Bracteoles:** (0) absent, (1) present; **(25.) Flower number:** (0) not a solitary flower, (1) a solitary axillary flower; **(26.) Flower resupination:** (0) not resupinate, (1) resupinate; **(27.) Uncinate trichomes:** (0) absent, (1) present on hypanthium and calyx; **(28.) Calyx lobe connation:** (0) less than half of total length, (1) more than half of total length; **(29.) Corolla insertion:** (0) erect in calyx, (1) oblique in calyx, (2) erect at base, but bending abruptly to appear oblique; **(30.) Floral anthocyanin color:** (0) absent, (1) pink to red, (2)

Appendix 2 (continued).

blue to purple; **(31.) Limb markings:** (0) without markings, (1) with markings; **(32.) Throat/tube interior markings:** (0) without markings, (1) with markings; **(33.) Throat constriction:** (0) not constricted (i.e., broader than tube), (1) constricted (i.e., distinctly narrower than tube); **(34.) Corolla tube length:** (0) nearly absent (tube shorter than width of mouth), (1) with a distinct, elongate tube (tube longer than width of mouth); **(35.) Upper corolla lobe margins:** (0) entire or nearly so, (1) distinctly toothed or fimbriate; **(36.) Lower corolla lobe margins:** (0) entire or nearly so, (1) distinctly toothed or fimbriate; **(37.) Corolla lobe indument 1:** (0) without glandular trichomes, (1) with short-stalked glandular trichomes, (2) with long-stalked glandular trichomes, (3) with barrel-shaped glandular trichomes; **(38.) Corolla lobe indument 2:** (0) without non-glandular trichomes, (1) with non-glandular trichomes; **(39.) Corolla interior indument:** (0) without glandular trichomes, (1) with glandular trichomes above anthers only, (2) with glandular trichomes throughout interior; **(40.) Spur:** (0) absent, (1) present; **(41.) Stamen insertion:** (0) at base of corolla tube, (1) on (distinctly adnate to) corolla tube; **(42.) Filament relative length:** (0) shorter than anther, (1) longer than anther; **(43.) Filament indument:** (0) without trichomes (glabrous), (1) with trichomes; **(44.) Stamen exsertion:** (0) not exserted, (1) distinctly exserted; **(45.) Anther coherence:** (0) not coherent, (1) coherent; **(46.) Anther thecae divergence:** (0) not divergent (i.e., more or less parallel); (1) divergent; **(47.) Thecae dehiscence:** (0) dehiscing by slits; (1) dehiscing by pores; **(48.) Staminode:** (0) dorsal stamen sterile (staminodial), (1) dorsal stamen fertile; **(49.) Ovary position:** (0) superior or nearly superior, (1) half inferior, (2) inferior or nearly inferior; **(50.) Nectary:** (0) absent; (1) annular; (2) divided into glands; **(51.) Style indument:** (0) lacking trichomes (glabrous), (1) with trichomes; **(52.) Stigma:** (0) not bilobed, (1) bilobed (*Kohleria*-type), (2) bilobed (*Diastema*-type); **(53.) Fruit texture:** (0) dry at maturity, (1) fleshy at maturity; **(54.) Fruit loculicidal dehiscence:** (0) not loculicidally dehiscent, (1) loculicidally dehiscent; **(55.) Fruit septicidal dehiscence:** (0) never with septicidal dehiscence, (1) can have secondary septicidal dehiscence; **(56.) Hypanthium splitting:** (0) not splitting at dehiscence, (1) splitting dorsally, (2) splitting irregularly; **(57.) Capsular trichomes:** (0) absent, (1) with trichomes on inside margins of valves; **(58.) Fruit shape:** (0) about as long as broad (e.g., globose or turbinate), (1) 1.5–2 times as long as broad (e.g., ovoid to ellipsoid), (2) 3–4+ times longer than broad (e.g., cylindric); **(59.) Fruit apex:** (0) not upturned, (1) pointed and upturned (rostrate); **(60.) Capsule costae:** (0) without prominent costae, (1) with prominent costae; **(61.) Seed shape:** (0) longer than broad (e.g., elliptic or fusiform), (1) about as broad as long (e.g., spherical, globose, to rhombic); **(62.) Seed appendages:** (0) without apical appendages, (1) with apical appendages.

Appendix 3. Morphological cladistic data matrix. Abbreviations "B" and "G" associated with *Gloxinia purpurascens* samples refer to disjunct populations from Bolivia and the Guianas, respectively. Numbers associated with undescribed species ("sp. nov.") refer to collection number designations in the voucher table and figures.

Achimenes antirrhina	3100100010	10?0000000	0101100011	1101111011	0100110011	1001001111 00
A. candida	3100100111	1000000000	0101000002	1101000010	0100100011	1001001111 00
A. cettoana	3100101000	00?0000000	0000100002	0?01000010	0100100011	1101001111 00
A. dulcis	3100100010	1000000000	0000100010	0001000010	0110110021	1001001110 00
A. erecta	3100101010	00?0000000	0001000001	0101000020	0100100021	1101001110 00
A. glabrata	3100100010	1000000000	0000100002	00010010?0	0100110011	100100???? ??
A. grandiflora	3100100010	10?0000000	0101100012	0101000011	0100110021	1101001110 00
A. longiflora	3100101000	0000000000	0000100002	0101000000	0100100011	1101001110 00
A. misera	3100100010	10?0001000	0000100002	0101000010	0100100011	0001001111 00
A. patens	3100100010	10?0000000	0101100012	0101010011	0100110021	1101001111 00
Anodiscus xanthophyllus	1210000010	20?0001001	1000100000	0001000100	0110000010	1001001110 00
Bellonia aspera	1020000010	0000011000	0101000000	0000000000	0001001110	100110111? 00
B. spinosa	1020000010	0000010000	0000100000	0000000000	0001001110	1001001111 00
Capanea affinis	?211010011	1000000000	0101010002	1011001010	0111110012	1001101001 01
C. grandiflora	1211010011	1000000000	0101010002	1101002020	0111110012	1001101001 01
Diastema affine	?200?00010	1000?01011	0000100002	1?010000?0	0100000012	0201010000 10
D. comiferum	?200100010	1000001011	0000100001	0101000010	0100100012	12?10????? ??
D. racemiferum	1200100010	1000001011	0000100002	1?01000000	0100100012	1211010111 10
D. scabrum	?200100010	1000001011	0000100002	1?01000000	0100100012	1?11010101 00
D. vexans	1200100010	1000001000	0001000002	1101001010	0100100012	12?101?1?? 00
Eucodonia andrieuxii	2100100010	1000000000	0000100012	0101000010	0100110011	0001001110 00
E. verticillata	2100100010	1000001000	0000100012	0?01000000	0100110011	100100?110 00
Gesneria acaulis	0020002000	2010011100	0101000001	0001100020	0100100021	0001000001 00
G. pedicellaris	?020002000	2020001100	0101000001	0001100020	0110100021	1001000001 00
G. pedunculosa	0020002010	1020011000	0100000000	0001001000	0111100021	0001100001 00
G. rupincola	?020002010	20?0001000	0111000001	0011100000	1110100011	1001000000 00
G. ventricosa	0020002010	2020010000	0101000001	0001101000	0101100021	1001100001 00
Gloxinia dodsonii	?200000010	1000001100	0000100012	0001000010	0110100021	1011010201 10
G. gymnostoma	1200110010	1000001000	0000100002	1101003000	0100100021	1001001111 01
G. sp. aff. *ichthyostoma*	?300100010	1000001000	000?100012	0?01110000	01001?0011	1101001111 10
G. lindeniana	1200110010	1000001000	0000100012	0001001010	0100100021	10110101?0 10
G. nematanthodes	1200110010	1000001000	0000100011	0111013000	0100100021	1001001111 01
G. perennis	1200100010	1000000001	0000100012	0001011010	0110100021	1001001111 00
G. planalta	?300100000	10?0001000	0001000012	0?01110?0	0100110011	0101001111 00
G. purpurascens [B]	?200110010	1000001000	0000100001	0111003000	0110100021	1001001111 00

Appendix 3 (continued).

G. purpurascens [G]	?400111010	1000001000	0000100001	0111003010	0100100021	1001001111	00
G. racemosa	?200100010	2010001001	0000100012	0101011010	0100100021	1011010000	10
G. reflexa	?200100011	10?0001000	0000000010	0001110010	0110100020	1011010201	10
G. sylvatica	1200111000	0000001000	0000000011	0111003000	0100100021	1001001111	01
Goyazia petraea	?300100100	01?0010000	0000100002	0101000010	0100100001	0101001111	00
G. rupicola	?300100100	01?0010000	0000100012	1101000000	0100100001	010100????	??
Heppiella ulmifolia	1210100010	1000001000	0001000001	0001000000	0101000011	1001001110	00
H. verticillata	?210100010	1000001000	0001000011	0001001100	0100000011	1001001110	00
H. viscida	1210100010	1000001000	0111000011	0001000000	0100000011	1001001110	00
Koellikeria erinoides	1200100010	1000000001	1000100012	0101011110	0110110011	0001001111	00
Kohleria allenii	1110101010	1000001001	0001100011	1101002000	0100100012	0101001110	00
K. amabilis	1200100010	0000001000	0001100011	1101002020	0110100012	1111010170	10
K. grandiflora	?200110011	1000001000	0101100021	1101002000	0110100022	1111010110	00
K. hirsuta	1210100010	1000001000	0001000001	1111002000	0100100012	1101001110	00
K. hondensis	?210100010	1000000000	0001000001	1111002000	0100100012	1101001110	00
K. peruviana	1210100010	1000000000	0101000001	1101002000	0100100012	1101001110	00
K. rugata	1100100010	1000001001	0000100011	1101002010	0100100012	1101001110	00
K. sp. nov. [2446]	?211110111	20?0001000	0000100021	0011001020	0110100022	1111010100	00
K. spicata	1110101010	1000101001	0001000011	1101002000	0110100012	1101001110	00
K. trianae	?210100010	1000000000	0101000001	1011002000	0100100012	1101001111	00
K. villosa	1200110010	1000001000	0001100021	0101000020	0110100012	1111010100	10
K. warszewiczii	1210100010	1000000000	0101000102	1111002000	0110100012	1101001110	00
Monopyle flava	?200100111	2000000001	0111001000	0001000010	01001?0020	0010010101	10
M. maxonii	1100100111	2000001001	0101001012	0001000000	0100110020	0011010201	10
M. puberula	?100100111	2000000001	0101001002	0101000010	0100110020	0011010201	10
Moussonia deppeana	3110000010	10?0001000	0101000001	1101110000	0110100011	1001001111	00
M. elegans	3110000010	10?0001000	0101000001	1101110000	0111100011	1001001111	00
M. septentrionalis	3110000010	10?0000000	0101000001	0001110070	0111100011	100100?110	??
Niphaea oblonga	3100100010	1000001000	0001000000	0000000020	0011000010	0001001110	00
N. peruviana	?200100010	10?0001100	0000100000	00000000?0	01001?0020	0011010200	10
Pearcea abunda	3200100010	1000000000	0101000021	0011003020	0110100012	1011000000	00
P. hypocyrtiflora	1200100010	1000101000	0001000021	0011001020	0110100012	1011000000	00
P. reticulata	?200100010	2000000000	0101000021	1?11001020	0100100012	1011010000	00
P. sprucei	?200100010	2000101000	0101000021	1?11001020	0100100012	1011010000	00
Pheidonocarpa corymbosa	0020000010	1020000000	0111000001	1001101000	0111100011	1001001110	00
Phinaea albolineata	?200100010	1000001000	0011000000	0001111010	0100110011	1011000000	00
P. divaricata	1200100010	1000001000	0001000000	000000???0	0100011010	0001000000	00
P. lacerata	?100100011	10?0000000	0001000000	0000000000	0100010010	0001000000	00
P. multiflora	1100100010	1000001000	0001000000	0000000000	0100111011	0011000000	00
P. sp. nov. [93-336]	?200100010	1000001000	0000000000	0000000000	0100011000	0001000000	00
Rhytidophyllum auriculatum	0020002000	3021101000	0111000001	0101100000	1111100021	0001100000	00
R. exsertum	?020002000	3020101000	0111000002	1101000000	1111100021	0001100000	00
R. tomentosum	0020002000	3021101000	0111000002	1101000000	1111100021	1001100000	00
Smithiantha aurantiaca	?100100010	0000000001	1000100001	0101000020	0100100011	1001001110	00
S. canarina	?100100010	0000000001	1000100000	0001000010	0110100011	1001001110	00
S. cinnabarina	2100100010	0000000001	1000100001	0101000020	0110100011	0001001111	00
S. multiflora	2100100010	0000000001	1000100000	0001000070	0110100011	1001001111	00
Solenophora calycosa	4110000011	20?0000000	0101000101	1001000000	0100100022	1010020001	00
S. tuerckheimiana	?110000011	20?0000000	0101000100	0001010070	0100100022	1010020000	??
S. tuxtlensis	4110000111	1010000000	0101000102	1101110010	0100100022	1010020100	00

Appendix 4. Comparison of bootstrap branch support of morphological MP trees under different weighting schemes. Letter designations (A, B, C, D, E, etc.) refer to the branches marked on Fig. 5. Tree abbreviations are as follows: EQ = all characters equal weight, FL = flower and inflorescence characters downweighted by half, FR = fruit and seed characters downweighted by half, LF = leaf and stem characters downweighted by half, and IN = indument characters downweighted by half.

Clade and clade members	EQ	FL	FR	LF	IN
A = *Gesneria acaulis*, *G. pedicellaris*, *G. pedunculosa*, *G. ventricosa*, *G. rupicola*, *Rhytidophyllum auriculatum*, *R. exsertum*, *R. tomentosum*, *Pheidonocarpa corymbosa*.	75	79	65	62	55
B = *Gesneria acaulis*, *G. pedicellaris*.	71	66	76	71	69
C = *Gesneria pedunculosa*, *G. ventricosa*.	54	52	---	51	54
D = *Rhytidophyllum auriculatum*, *R. exsertum*, *R. tomentosum*.	96	96	99	95	98
E = *Rhytidophyllum exsertum*, *R. tomentosum*.	70	---	81	75	80
F = *Gesneria acaulis*, *G. pedicellaris*, *G. pedunculosa*, *G. ventricosa*, *G. rupicola*, *Rhytidophyllum auriculatum*, *R. exsertum*, *R. tomentosum*.	---	---	71	---	82
G = *Solenophora calycosa*, *S. tuerckheimiana*, *S. tuxtlensis*.	84	92	81	92	88
H = *Pearcea reticulata*, *P. sprucei*.	71	66	70	78	72
I = *Moussonia deppeana*, *M. elegans*.	67	77	56	---	74

Appendix 4 (continued).

Clade and clade members	EQ	FL	FR	LF	IN
J = *Monopyle flava, M. maxonii, M. puberula.*	92	90	94	92	89
K = *Monopyle maxonii, M. puberula.*	---	---	60	66	---
L = *Kohleria allenii, K. rugata, K. spicata.*	58	---	53	---	58
M = *Kohleria allenii, K. spicata.*	---	68	63	---	---
N = *Gloxinia gymnostoma, G. nematanthodes, G. sylvatica, G. purpurascens* (Bolivia), *G. purpurascens* (Guianas).	76	78	66	76	70
O = *Gloxinia gymnostoma, G. nematanthodes, G. sylvatica.*	---	53	---	---	---
P = *Gloxinia nematanthodes, G. sylvatica.*	55	53	54	66	50
Q = *Gloxinia nematanthodes, G. sylvatica, G. purpurascens* (Bolivia), *G. purpurascens* (Guianas).	---	---	55	---	---
R = *Diastema affine, D. racemiferum, D. scabrum, D. comiferum.*	54	---	56	---	---
S = *Diastema affine, D. racemiferum, D. scabrum.*	58	---	64	---	55
T = *Diastema affine, D. racemiferum.*	---	---	55	---	---
U = *Capanea affinis, C. grandiflora.*	100	100	99	100	100
V = *Smithiantha aurantiaca, S. canarina, S. multiflora, S. cinnabarina.*	---	51	56	---	53
W = *Smithiantha canarina, S. multiflora.*	51	---	56	---	---
X = *Achimenes cettoana, A. longiflora, Goyazia petraea, G. rupicola.*	---	---	53	---	---
Y = *Goyazia petraea, G. rupicola.*	95	97	98	90	98
Z = *Bellonia aspera, B. spinosa.*	97	99	97	93	92
AA = *Niphaea oblonga, Bellonia aspera, B. spinosa.*	---	---	53	55	55
BB = *Achimenes grandiflora, A. patens.*	66	---	70	62	65
CC = *Gloxinia* sp. aff. *ichthyostoma, G. planalta.*	72	55	80	63	75
DD = *Gloxinia perennis, Koellikeria erinoides.*	63	70	---	63	57
EE = *Heppiella verticillata, H. viscida.*	---	---	57	---	53
FF = *Kohleria* sp., *Pearcea abunda, P. reticulata, P. sprucei.*	---	---	53	---	---