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Independent Origin of Radial Floral Symmetry in the Gloxinieae (Gesnerioideae: Gesneriaceae) is Supported by the Rediscovery of *Phinaea pulchella* in Cuba

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Abstract*—Phinaea*, in the currently accepted circumscription, is a genus in the flowering plant family Gesneriaceae with three widely disjunct species. These species are known from small populations in Mexico, northern South America, and the West Indies (Cuba and Haiti), respectively. *Phinaea pulchella* is one of the few members of the tribe Gloxinieae that occurs naturally in the West Indies and it is the only member of the tribe endemic to that region. It was rediscovered in Cuba in 2008, more than fifty years after it was last documented. Results from molecular data generated from the nrDNA ITS and cpDNA *trnL-F* regions strongly support that *P. pulchella* does not group with other *Phinaea* species and instead shares a recent common ancestor with *Diastema vexans* in a clade that is sister to *Pearcea* and *Kohleria*. The phylogenetic placement of *P. pulchella* suggests that radial floral symmetry and buzz-pollination is autapmorphic in this taxon. Our results strongly support convergence of radial symmetry and associated characters with buzz-pollination in the following taxa in the tribe Gloxinieae: *Niphaea*, *Phinaea* s. s., *Phinaea pulchella*, and *Amalophyllon*. New generic circumscriptions based on the results presented here are not suggested until more complete taxon sampling includes additional species currently recognized in *Amalophyllon*.

***Keywords*—**Buzz-pollination, floral symmetry, Gesneriaceae, Gloxinieae, *Phinaea*.

Tribe Gloxinieae (Gesneriaceae) is a morphologically diverse clade that presently includes 20 genera and around 170 species (Burtt and Wiehler 1995; Weber 2004; Skog and Boggan 2006). This tribe is an exclusively New World clade of the subfamily Gesnerioideae (Zimmer et al. 2002; Smith et al. 2004a, b; Roalson et al. 2005a, b, 2008). Recent morphological and molecular studies (Smith et al. 2004a, b; Roalson et al. 2005a, b; Boggan et al. 2008) of the tribe Gloxinieae resulted in the publication of four new genera and one new tribe (Roalson et al. 2005b). In addition to the new genera, available generic names were reinstated to reflect strongly supported phylogenies (Roalson et al. 2005a; Boggan et al. 2008).

Of particular interest in the recent phylogenetic analyses was the diphyletic nature of *Phinaea*, and the multiple independent origins of rotate corollas in the Gloxinieae (Smith et al. 2004a, b; Roalson et al. 2005a). The traditional circumscription of *Phinaea* was shown to represent two independent lineages, neither of which is closely related to another genus with a rotate corolla: *Niphaea* (Smith et al. 2004a, b; Roalson et al. 2005a, b). Based on these results, Boggan et al. (2008) recognized two genera, *Phinaea* and *Amalophyllon*, to correspond to the two phylogenetic lineages previously considered within *Phinaea*.

Based on a recent review (Boggan et al. 2008) the following three species are recognized in *Phinaea*: *P. albolineata* (Hook.) Benth. ex Hemsl., *P. multiflora* C. V. Morton, and *P. pulchella* (Griseb.) C. V. Morton. The latter also has a variety (*P. pulchella* var. *domingensis* (Urb. & Ekman) C. V. Morton) that is only known from one collection made from Haiti in 1927. These three species have widely disjunct geographical distributions. *Phinaea albolineata* is known from two areas in South America (Norte de Santander in Colombia and Pará in Brazil) and *P. multiflora* is widespread in Mexico (Guerrero, Jalisco, Michoacan, Nayarit, Oaxaca, and Sinaloa). *Phinaea pulchella* is known from Cuba and Haiti. There are no known collections of *Phinaea* in Central America from areas south of Mexico and north of Colombia.

The three Gloxinieae genera with rotate to subrotate white corollas were recognized in early classifications in the tribe Bellonieae with *Bellonia* L. (Fritsch 1893–1894; Fig. 1A), which is now known to belong to the tribe Gesnerieae (Roalson et al. 2005a, 2008). Smith et al. (2004a) and Roalson et al. (2005a, 2008) suggest that in the New World Gesneriaceae there have been four independent origins of subrotate white corollas (three in tribe Gloxinieae and one in tribe Gesnerieae) and associated characters of a vibratory or buzz-pollination syndrome. It should be noted that *Bellonia* is the only New World genus in the Gesneriaceae with an androecium of five anthers and radially symmetrical corollas. All other members of the New World Gesneriaceae with radially symmetrical corollas have androecia with four anthers.

Subrotate or radially symmetrical corollas are otherwise rare in the New World members of the Gesneriaceae subfamily Gesnerioideae and these are likely not associated with vibratory pollination. The presence of powdery, nonsticky pollen, which is indicative of buzz-pollination was not evaluated in the present study. Cultivated species of *Sinningia speciosa* with peloric mutants can have radially symmetrical flowers (Burtt 1970; Coen and Nugent 1994; Möller et al. 1999; Citerne et al. 2000). Otherwise, the only other genus in the New World Gesneriaceae with subrotate corollas is *Napeanthus* (Fig. 1E), a small terrestrial herb with a basal rosette of leaves. The corolla tube is short and the flower symmetry is radial to weakly bilabiate. Little is known about the pollination biology of this small genus of 18 species because they are difficult to locate, not well represented in herbaria, and their flowers are extremely ephemeral. The anthers of *Napeanthus* are orbicular to oblong and dehisce by longitudinal slits (Fig. 1E). The absence of poricidal dehiscence is one indication that the flowers lack buzz-pollination even though the corolla is subrotate. It is not currently known if the pollen grains of *Napeanthus* are sticky or nonsticky (powdery) which would allow a more accurate assessment of how the flowers are exploited by pollinators.

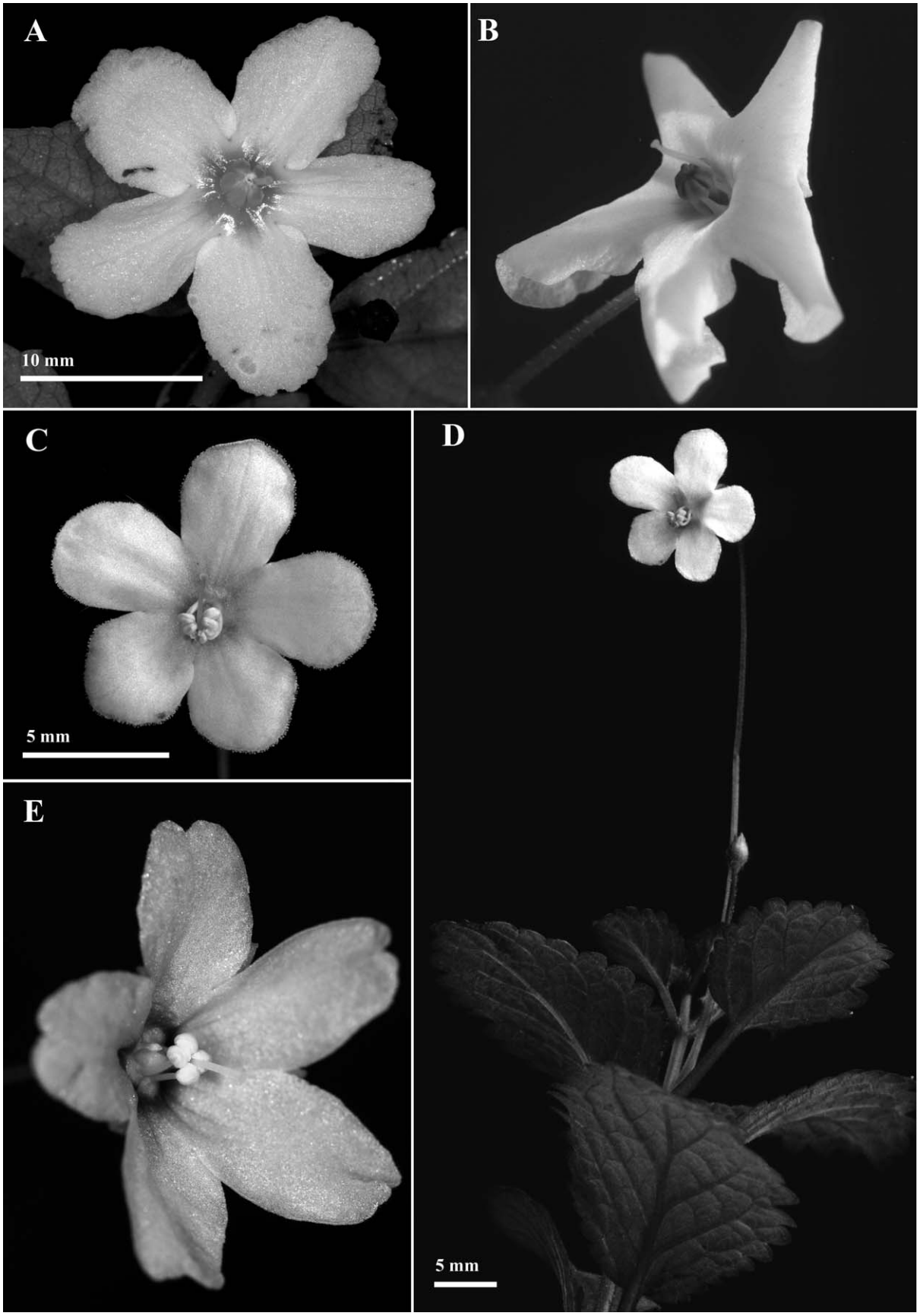

Fig. 1. Images of New World Gesneriaceae with radially symmetrical flowers. A. *Bellonia spinosa*. B. *Niphaea oblonga*, approximate flower diameter = 10 mm. C. *Amalophyllon divaricatum*. D. *Amalophyllon divaricatum*. E. *Napeanthus* sp., approximate flower diameter =10 mm. (Voucher specimens at US: A. *J. L. Clark 10573*; B: United States Botany Research Greenhouse accession number 1978–354; C & D: *J. L. Clark 8840*; E: *J. L. Clark et al. 10345*; Photos: A & E by John L. Clark; B by Leslie Brothers, Smithsonian Institution Department of Botany; C & D by James Di Loreto, Smithsonian Institution NMNH Imaging).

Subrotate and radially symmetrical corollas in the Old World members of the Gesneriaceae subfamily Cyrtandroideae are found in the genera *Conandron* Sieb. & Zucc., *Paraboea* (Clarke) Ridley, *Ramonda* Rich., *Saintpaulia* H. A. Wendl., *Bournea* Oliv. *Boea* Comm. ex Lam., *Didymocarpus* Wall., and *Tengia* Chun. *Saintpaulia* is a commonly cultivated genus of six (Darbyshire 2006) to 20 species (Burtt 1958) that is native to tropical Africa and has corollas that are weakly bilabiate to radial and is associated with buzz-pollination (Vogel 1978; Dafni 1992; Harrison et al. 1999). Radially symmetrical corollas with an androecium of five anthers are found only in *Ramonda*, *Bournea*, *Conandron*, and *Tengia*. The other genera mentioned above have radially symmetrical corollas with androecia of four or two anthers.

Phinaea pulchella is only known from the Caribbean islands of Haiti and Cuba. The type collection was made by Charles Wright (*C. Wright 3069*) from the Loma de Rangel region in western Cuba in 1863 (Howard 1988). This locally endemic species is only known from seventeen collections, most of which were made in the early 20th century. It was collected once in Haiti in 1927 (*P. pulchella* var. *domingensis*) and had not been collected in Cuba for over fifty years. In 2008, this species was rediscovered by a team of botanists organized through the University of Alabama Cuba Initiative in the vicinity of the type locality (*J. L. Clark et al. 10583*; Fig. 2). This was the first collection of the species since its last collection in 1955 from the Viñales region of western Cuba.

Phinaea pulchella is a small herb restricted to limestone cliffs. The vertical cliffs where this species occurs are usually dry with sporadically distributed moist areas with small clusters of mosses and herbs. It is on these moist areas that *Phinaea pulchella* was found to be locally abundant. This species shares a suite of characters with other members of *Phinaea* including erect pedicels in fruit (Fig. 2A), valves fleshy at dehiscence and opening broadly (Fig. 2B), sticky seeds adhering to the valves (Fig. 2B), nectary annular, and corolla uniformly white, with a strongly reduced tube and large subrotate limb (Fig. 2C, D; Boggan et al. 2008). However, *Phinaea pulchella* is the only species of *Phinaea* known from the Caribbean (the two other species are Central and South American in distribution), and is the only species in the tribe Gloxinieae endemic to the Caribbean (Boggan et al. 2008).

Phylogenetic analyses of relationships in the Gloxinieae have in several instances demonstrated morphological convergence and problems with generic circumscription, including the separation of *Amalophyllon* from *Phinaea* (Boggan et al. 2008), and the disentanglement of *Gloxinia*, *Gloxiniella*, *Gloxiniopsis*, *Goyazia*, *Mandirola*, *Nomopyle*, and *Seemannia* (Roalson et al. 2005a, b). Given the difficulties in morphological definition of genera in the Gloxinieae and the fact that *Phinaea pulchella* has such a disjunct distribution from the rest of *Phinaea*, we here explored the phylogenetic origins of *P. pulchella* using nrDNA and cpDNA data sets to retest the monophyly of *Phinaea*, and determine from what geographic area this narrow endemic originated.

Materials and Methods

Taxon Sampling—*Phinaea pulchella* was sequenced for the nrDNA ITS regions and the cpDNA *trnL-F* region (*trnL* intron and *trnL-F* intergenic spacer). These data were added to the matrix generated by Roalson et al. (2005a). In total, sixty-two species were analyzed, including six species from the tribe Gesnerieae, the sister group of the Gloxinieae. The six species included in the analysis from the tribe Gesnerieae were *Bellonia spinosa*, *Gesneria acualis*, *G. pedunculosa*, *Pheidonocarpa corymbosa*, *Rhytidophyllum auriculatum*, and *R. exsertum*.

DNA Extraction, Amplification, and Sequencing—Genomic DNA from *Phinaea pulchella* was isolated using the Qiagen DNeasyTM DNA isolation kit (Qiagen, Valencia, California). The template of the nrDNA ITS region was prepared using the primers ITS4 and ITSL (Suh et al. 1993). Templates of the cpDNA *trnL-F* region were amplified using the primers *trnLc* and *trnLf* (Taberlet et al. 1991).

Polymerase chain reaction (PCR) amplifications followed the procedures described by Baldwin et al. (1995) utilizing Taq DNA polymerase (Promega, Madison, Wisconsin). To reduce within-strand base pairing that can result in interference with Taq polymerase activity, we found it useful to include 5% DMSO and 16% Betaine in PCR reactions. A hot start was performed by raising the temperature of the reaction to 94°C and then followed by the addition of Taq DNA Polymerase. The PCR products were then electrophoresed using a 1.0% agarose gel in 1 × SB (pH 8) buffer, stained with ethidium bromide to confirm a single product, and purified following Johnson and Soltis (1995) clean-up protocol. Direct cycle sequencing of purified template DNA followed the manufacturer's specifications, using the ABI Prism BigDye™ terminator cycle sequencing ready reaction kit (PE Bio-systems, Foster City, California). The ITS cycle sequencing was carried out with the two initial PCR primers, (ITS4 and ITSL) and the internal primers, (ITS2 and ITS3). The same was performed for the *trnL-F* regions using primers *trnLc*, *trnLd*, *trnLe*, and *trnLf*. Chromatograms were proofed, edited, and contigs assembled using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan).

Alignment and Phylogenetic Analyses—Sequences were aligned using ClustalW2 (Larkin et al. 2007) and then imported into the program SeAl version 2.0a11 Carbon (Rambaut 1996) multiple sequence editor for the final alignment. Because the sequences were not divergent, it was possible to make minor adjustments so that overlapping gaps were minimized. This approach allowed for single-site and multiple-site gaps to be treated with equal weight (Simmons and Ochoterena 2000). Tree searches were carried out with gaps as missing data in the alignment, but indels of constant length were incorporated by scoring as a presence/absence character. Phylogenetic data matrices are available in Nexus format from TreeBASE (study number SN4938).

Phylogenetic tree searches were performed using three approaches: maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). These analyses were run on two different data sets: The combined ITS/*trnL-F* matrix and the ITS/*trnL-F* + gaps matrix. As gaps cannot be easily incorporated into ML analyses, the MP and BI analyses included gaps and the ML analysis did not.

The MP analysis was performed to completion using a two stage heuristic search in PAUP*4.0b10 (Swofford 2002). The first stage of the analysis was done using the following settings: 10,000 random addition cycles, holding 10 trees at each step; tree bisection-reconstruction (TBR) branch swapping with no more than 10 trees saved for each rep; MULTREES option not in effect. The second stage of the analysis was performed on all trees in memory with the same settings, but with the MULTREES option in effect. Other searches were conducted, but did not find shorter trees using the settings above with the following changes: 10 random addition cycles limited to 10,000 trees of equal length for each of the replicates; 1,000 random addition cycles limited to 100 trees of equal length for each of the replicates.

Additional tree searches were done using the parsimony ratchet analysis with NONA (Goloboff 1999) and Winclada (Nixon 2002). Five separate tree searches were conducted using the following settings: 1,000 iterations per search, one tree held for each iteration, 160 characters sampled (10% of the total), and amb = poly- (only considers unambiguous support). Five multiple ratchet searches were performed in WinClada as suggested by Nixon (1999) since the ratchet option can sometimes get stuck on suboptimal "islands" and it is therefore better to perform more searches with fewer iterations than one larger search with more iterations.

Clade robustness was evaluated in PAUP* using the bootstrap (Felsenstein 1985). The bootstrap analysis used 1,000 heuristic bootstrap replicates with the following settings: 10 random addition cycles; tree bisection-reconstruction (TBR) branch swapping with no more than 10 trees saved for each replicate; and the multiple trees option not in effect.

Maximum likelihood analyses employed heuristic searches (TBR branch swapping). Clade support was estimated using 100 heuristic bootstrap replicates (100 random addition cycles and 1,000 total rearrangements per replicate, TBR branch swapping (Felsenstein 1985; Hillis and Bull 1993). Maximum likelihood analysis of the ITS data set employed the Tamura and Nei (1993) TrN model with empirical base frequencies, proportion of invariant sites (I), and gamma shape (G) parameters (six substitution types: A/C- 1.0000, A/G- 2.6934, A/T- 1.0000, C/G- 1.0000,

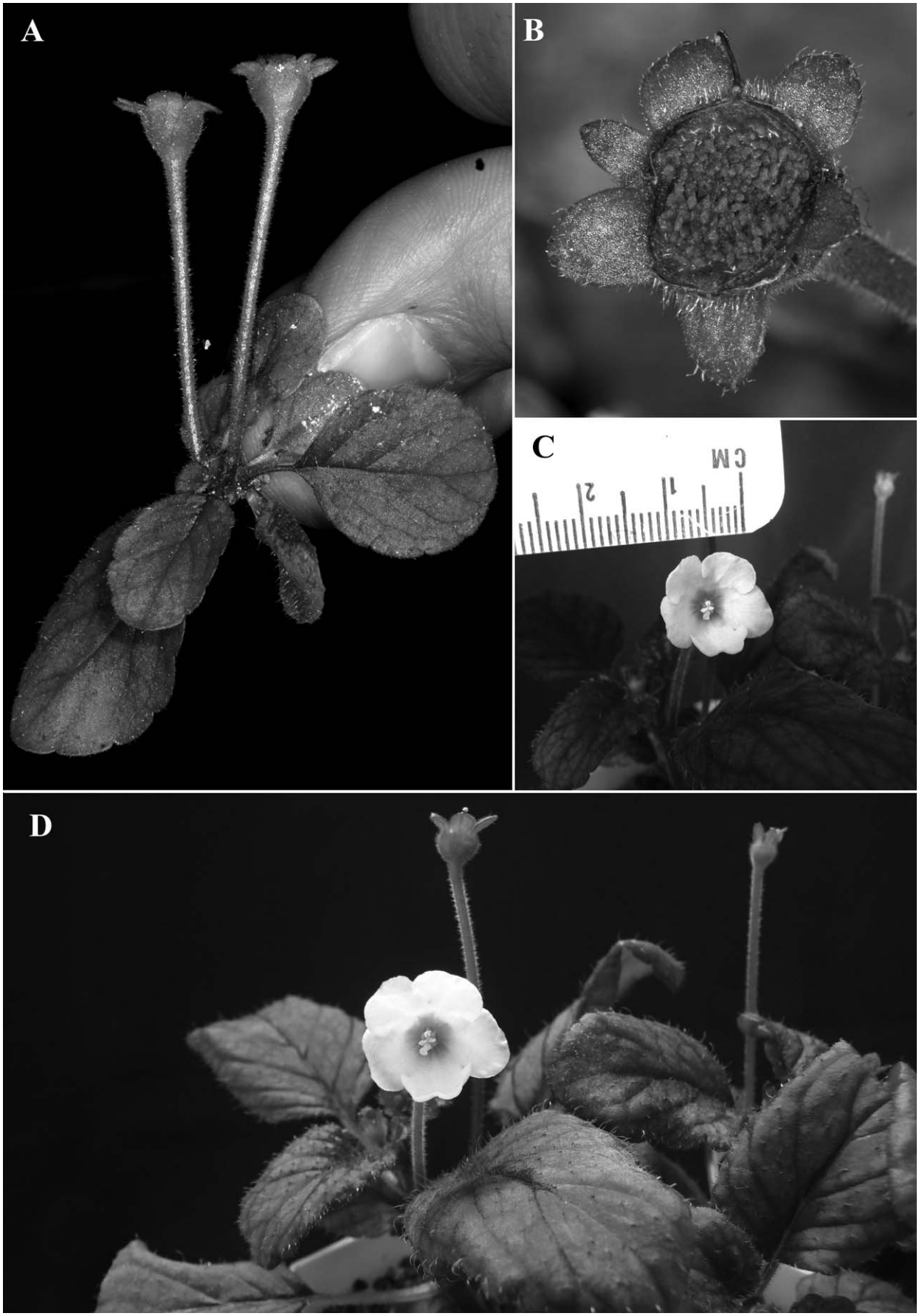

Fig. 2. Images of *Phinaea pulchella*. A. Habit showing erect pedicels. B. Capsule with broadly spreading dehiscence and seeds adhering to valves. C. Rotate Flower. D. Habit showing flower. (A-D *J. L. Clark et al. 105830* (US); Photos: A & B by John L. Clark; C & D by Nancy Kast).

C/T- 4.5613, G/T- 1.0000; base frequencies: A- 0.3059, C- 0.1941, G- 0.1967, T- 0.3033; I = 0.4980; G = 0.6735). This model was chosen based on the results of analysis using DT_ModSel (Minin et al. 2003). The DT_ModSel analysis uses a Bayesian information criterion to select a model using branch-length error as a performance measure in a decision theory framework that also includes a penalty for model overfitting.

Bayesian inference analyses were performed using MrBayes v. 3.1 (Huelsenbeck and Ronquist 2001). Three partitions to the data matrix were modeled independently: ITS, *trnL-F*, and gap characters. Model selection was conducted using DT-ModSel (Minin et al. 2003), with the following models chosen: ITS – SYM + I + G (Zharkikh 1994); and *trnL-F* – K81uf + G (Kimura 1981); and gap characters, standard discrete model as implemented in MrBayes. Four chains were run for 10,000,000 generations each, and sampled every 10,000 generations. Multiple independent BI analyses were run to test for convergence and mixing.

***Tests of Alternative Topologies*—**Given low support at some nodes deep in the trees, two different methods were used to assess whether other topologies could be statistically rejected: the Shimodaira-Hasegawa (SH) test, and Bayesian confidence interval. The SH test (Shimodaira and Hasegawa 1999) was implemented in PAUP* comparing constraint ML topologies with the best ML tree using 5,000 RELL bootstraps. The posterior distribution of trees from the Bayesian inference analysis was assessed for the proportion of trees in the posterior distribution that had the partitions of interest. As the posterior distribution should reflect a statistical confidence interval, the proportion of trees with the partition to be tested can be compared with the 95% confidence limits to give an indication of whether a particular partition can be statistically rejected. Eight alternative topologies were compared to the ML or Bayesian topologies to test whether four separate origins of rotate corollas suggested by the ML tree could be rejected. These were (1) monophyly of all rotate Gloxinieae with rotate corollas ("rotate test"; monophyly of *Amalophyllon clarkii*, *A. divaricatum*, *Niphaea oblonga*, *Phinaea albolineata*, *P. multiflora*, and *P. pulchella*); (2) monophyly of all rotate corolla Gloxinieae + *Diastema vexans* ("rotateD test"; monophyly of *Amalophyllon clarkii*, *A. divaricatum*, *Diastema vexans*, *Niphaea oblonga*, *Phinaea albolineata*, *P. multiflora*, and *P. pulchella*); (3) monophyly of *Amalophyllon* + *Phinaea* ("AP test"; monophyly of *Amalophyllon clarkii*, *A. divaricatum*, *Phinaea albolineata*, *P. multiflora*, and *P. pulchella*); (4) monophyly of *Amalophyllon* + *Phinaea* + *Diastema vexans* ("APD test"; monophyly of *Amalophyllon clarkii*, *A. divaricatum*, *Diastema vexans*, *Phinaea albolineata*, *P. multiflora*, and *P. pulchella*); (5) monophyly of *Phinaea* ("P test"; monophyly of *Phinaea albolineata*, *P. multiflora*, and *P. pulchella*); (6) monophyly of *Phinaea* + *Diastema vexans* ("PD test"; monophyly of *Diastema vexans*, *Phinaea albolineata*, *P. multiflora*, and *P. pulchella*); (7) monophyly of *Amalophyllon* + *Phinaea pulchella* ("APp test"; monophyly of *Amalophyllon clarkii*, *A. divaricatum*, and *Phinaea pulchella*); and (8) monophyly of *Amalophyllon* + *Phinaea pulchella* + *Diastema vexans* ("APpD test"; monophyly of *Amalophyllon clarkii*, *A. divaricatum*, *Diastema vexans*, and *Phinaea pulchella*). The "rotate test" was used to test whether the four rotate corolla lineages could be rejected as monophyletic. The "rotateD test" was conducted because the strong support for the placement of *D. vexans* with *P. pulchella* might negatively affect the rotate test; so, this test would determine whether we could reject monophyly of the all of the rotate corolla lineages plus *D. vexans* together. The AP and APD tests test for the monophyly of *Amalophyllon* and *Phinaea* with or without *D. vexans*, but excluding *Niphaea*, given the strong branch support separating *Niphaea* from the other rotate corolla species. The P, PD, APp, and APpD tests similarly test for statistical exclusion of monophyly of various combinations of rotate corolla species with or without the inclusion of *D. vexans*. These tests together explore the support for all of the most obvious possibilities for the number of origins of rotate corollas.

Results

***DNA Sequencing and Alignment*—**The four ITS sequencing primers produced overlapping fragments that collectively covered the entire spacer and 5.8S rDNA regions along both strands. The aligned ITS data matrix was 652 base pairs (bp) long with 427 (65%) variable sites, of which 219 (34%) were parsimony informative. The length of the unaligned sequences varied from 608–622 bp. The outgroups of the analysis contributed 15 of the 219 parsimony informative characters. There were no ambiguously aligned sites excluded from the analysis. The aligned data matrix contained six parsimony informative indels ranging from two to five bp in length and the mean pairwise divergence for the entire ITS region was 8.7%.

The two *trnL-F* sequencing primers produced overlapping fragments that collectively covered the entire *trnL* intron and the *trnL-trnF* intergenic spacer regions along both strands. The aligned *trnL-F* data matrix was 946 bp long with 289 variable sites (31%), of which 51 (5%) were parsimony informative. The length of the unaligned complete sequences varied from 626–908 bp. The outgroups of the analysis contributed seven of the 51 parsimony informative characters. There were no ambiguously aligned sites excluded from the analysis. The aligned data matrix contained four parsimony informative indels ranging from two to 17 bp in length and the mean pairwise divergence for the entire *trnL-F* region was 1.4%.

***Phylogenetic Analyses*—**The parsimony analysis of the combined ITS and *trnL-F* data resulted in 19 most parsimonious trees (length = 1,205 steps, consistency index [CI] = 0.54, retention index [RI] = 0.68, rescaled consistency index [RC] = 0.37). Figure 3 shows the strict consensus of these trees.

The clade containing *Phinaea pulchella*, *Diastema vexans*, *Pearcea* (four species), and *Kohleria* (12 species) is strongly supported with a bootstrap value of 96% (Fig. 3). The other two species of *Phinaea* (*P. multiflora* and *P. albolineata*) are strongly supported as sister species (bootstrap = 90%) in a clade that is sister to *Monopyle* (two species).

The individual analysis of the *trnL-F* data did not result in a well-resolved phylogeny (results not presented here). The combined analysis of the two datasets provided more resolution over the analysis of individual datasets. Thus, a combined analysis for the two datasets presented here is considered to be the most appropriate representation of phylogenetic signals based on lack of apparent data conflict and the inherent benefits of a total evidence approach (Kluge 1989; Bruneau et al. 1995; Nixon and Carpenter 1996; Graham et al. 1998).

The ML analysis resulted in a single tree (-lnL = 9,091.97540; Fig. 4), and ML bootstrap results are presented on branches with a frequency greater than or equal to 50% (Fig. 4). Two independent BI analyses resulted in identical posterior distributions of trees as measured by posterior probabilities on the majority rule consensus trees, suggesting that convergence and mixing were occurring and that the posterior probabilities reasonably represent the posterior distribution of trees. The posterior probabilities (PP) from one of the analyses are presented on the ML tree as a proportion (Fig. 4). Results from the tests of alternative topologies strongly reject all of the alternative topologies tested (Table 1).

Topologies presented here suggest that *Phinaea* (sensu Boggan et al. 2008) is polyphyletic with *Phinaea pulchella* weakly supported as sister to *Diastema vexans* (ML; Fig. 4), in a grade with *D. vexans*, *Pearcea*, and *Kohleria* (BI; Fig. 4), or in an unresolved position (MP; Fig. 3). Two other species of *Phinaea* are strongly supported as forming a clade and also strongly supported as the sister lineage to *Monopyle* (Figs. 3–4).

Discussion

The results presented here strongly reject the monophyly of *Phinaea*, as currently circumscribed by Boggan et al. (2008) (Figs. 3–4; Table 1). *Phinaea pulchella* is neither closely related to two remaining species of *Phinaea*, nor to other Gesneriaceae native to the Caribbean. Instead, it is weakly supported as the sister group to *Diastema vexans* (Fig. 4; a poorly known terrestrial herb from wet cliff faces in the Valle del Cauca region

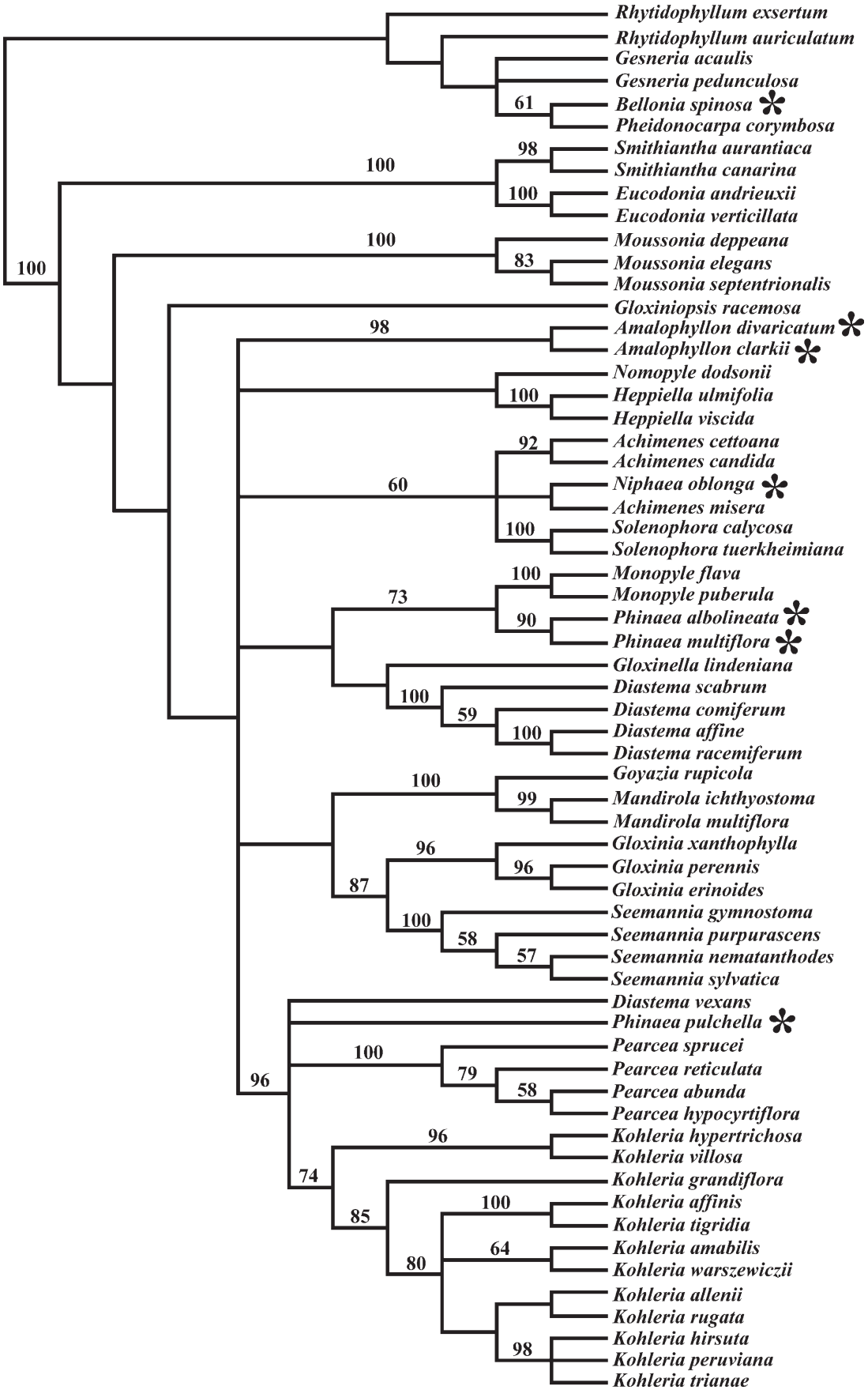

FIG. 3. Strict consensus of nine most parsimonious trees (length = 1,205 steps) from parsimony analysis of nrDNA ITS and cpDNA *trnL-F* data sets (CI = 0.54, RI = 0.68). Tree rooted with members from the tribe Gesnerieae (*Rhytidophyllum exsertum*, *R. auriculatum*, *Gesneria acaulis*, *G. pedunculosa*, *Bellonia spinosa*, and *Pheidonocarpa corymbosa*). Numbers above branches are bootstrap values (>50%). asterisk = taxa with radially symmetrical flowers.

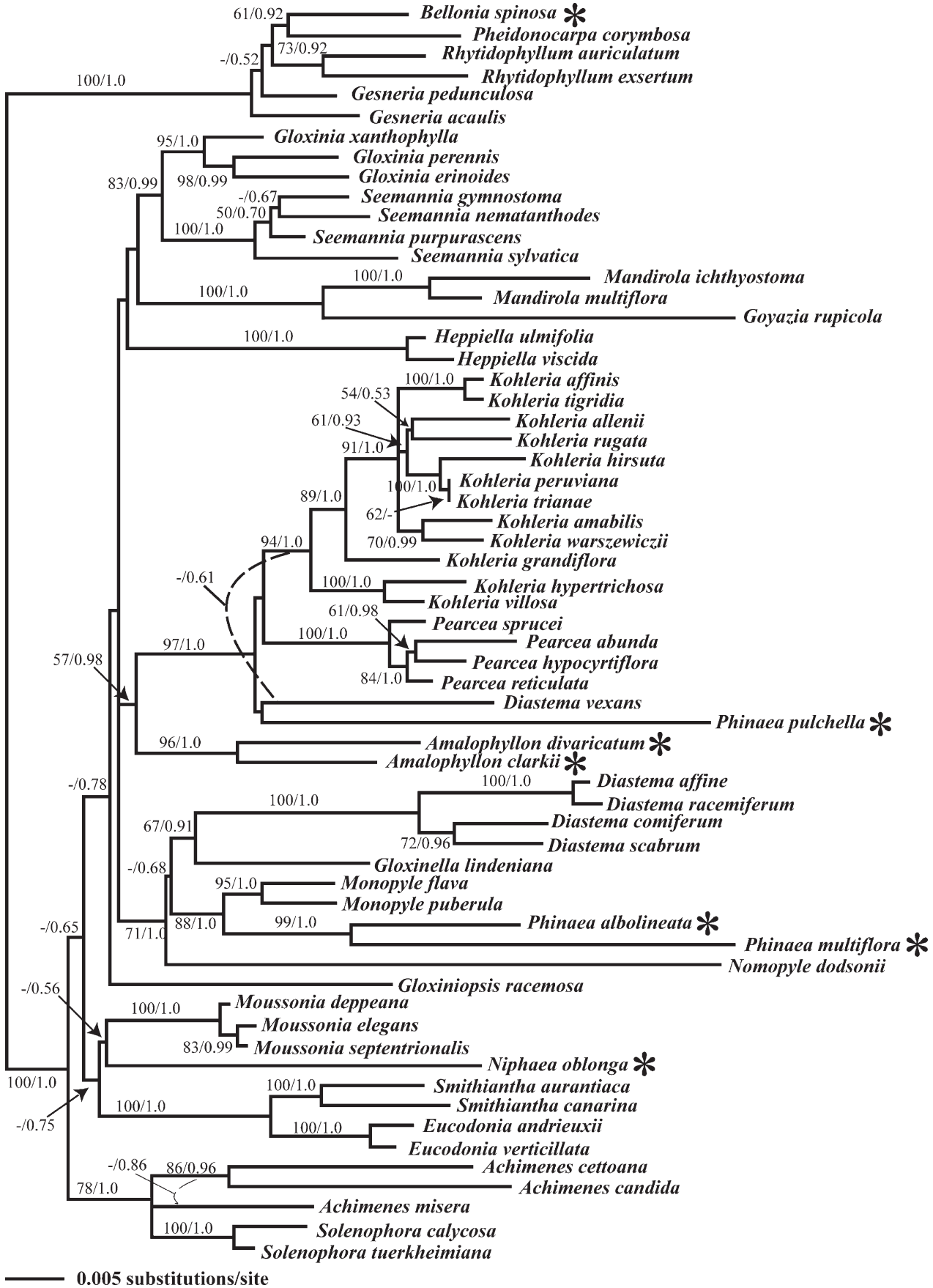

FIG. 4. Maximum likelihood phylogram from parsimony analysis of nrDNA ITS and cpDNA *trnL-F* data sets. Numbers at each node are ML bootstrap and Bayesian posterior probability values, respectively. Where the Bayesian majority rule consensus differs from the ML tree, the Bayesian topology is presented as an alternative using dashed lines connecting branches. asterisk = taxa with radially symmetrical flowers.

Table 1. Alternative topology tests for monophyly of rotate corolla lineages. Significant results are noted by asterisks.

Test	Best ML tree (-ln L)	Constraint tree (-ln L)	Difference (-ln L)	SH Test *p* value	Proportion of trees in the posterior distribution	Confidence interval
rotate	9,091.97540	9,177.44915	85.47375	0.0000*	0/800	0.000*
rotateD	-	9,221.77313	129.79773	0.0000*	0/800	0.000*
AP	-	9,163.27816	71.30276	0.0000*	0/800	0.000*
APD	-	9,209.77783	117.80243	0.0000*	0/800	0.000*
P	-	9,175.97813	84.00273	0.0002*	0/800	0.000*
PD	-	9,214.99617	123.02077	0.0000*	0/800	0.000*
APp	-	9,127.15982	35.18442	0.0032*	0/800	0.000*
APpD	-	9,155.84769	63.87228	0.0018*	0/800	0.000*

of Colombia), or in a grade with *D. vexans* leading to the *Pearcea* and *Kohleria* clades (Fig. 4). While the precise relationship of *Phinaea pulchella* and *Diastema vexans* is as yet unclear, there is strong evidence that *P. pulchella* does not form a clade with any of the other rotate corolla species of Gloxinieae, and this idea can be statistically rejected with or without the inclusion of *D. vexans* (Table 1). This strongly suggests that within the Gloxinieae there have been four independent origins of rotate corollas: in *Amalophyllon*, *Niphaea*, *Phinaea*, and *Phinaea pulchella*. Some portions of trees sampled in some of the analyses also suggest possible associations of some of the other rotate corolla lineages with the *Pearcea*/*Kohleria* clade (Figs. 3–4). The ML and BI analyses place the *Amalophyllon* clade as sister to the *P. pulchella*/*D. vexans*/*Pearcea*/*Kohleria* clade (Fig. 4). However, there is not consistent support for this association, it is not present in the MP analysis, 57% bootstrap in the ML analysis, and a 0.98 PP in the BI analysis, and there is no evidence that these lineages form a clade with *P. pulchella* (Table 1).

The few species from the Gloxinieae that occur in the Caribbean include *Achimenes erecta* (Lam.) H. P. Fuchs (Hispaniola, Jamaica), *Achimenes longiflora* DC. (Dominica, Guadeloupe, Jamaica, Martinique), *Gloxinia perennis* (L.) Fritsch (widespread), and several species of *Kohleria*. None of these species are closely related to *Phinaea pulchella*. Furthermore, most of the Gloxinieae were probably introduced from cultivated material and are also widely distributed in Central America. *Phinaea pulchella* is the only member of the tribe Gloxinieae that is endemic to the Caribbean and is most likely an independent colonization from South America.

Independent Origins of Vibratory or Buzz-Pollination—The recurrence of major shifts in pollination syndrome has been found previously in the tribes Gesnerieae (Martén-Rodríguez et al. 2010) and Gloxinieae (Cronk and Möller 1997; Smith et al. 2004a, b; Roalson et al. 2005a). Vibratory pollen collection (buzz pollination) by bumblebees is often associated with flowers that produce little or no nectar (Buchmann 1983; Proctor et al. 1996). In groups where little nectar is produced, the reward is often pollen that is nonsticky (powdery), which is released through pores at the tips of the anthers by vibrations generated from the frequency caused by the movement of insect wings. Vibratory pollen release and apical pores are therefore the norm in most anthers with apical poricidal anther dehiscence (Endress 1994, 1997; Garcia and Barboza 2006; Marazzi et al. 2007). Most likely, the poricidal anthers in most radially symmetrical flowers of Gesneriaceae (e.g. *Phinaea*, *Niphaea*, *Amalophyllon*, and *Bellonia*) are associated with this type of pollination syndrome. Field observations of vibratory pollen release have been verified in *Bellonia spinosa* (Silvana Martén-Rodríguez, pers. comm.), but have yet to be directly observed in other Gesneriaceae with rotate corollas.

Several other plant groups are adapted to vibratory pollen collection such as *Solanum* (Solanaceae; Vogel 1978; Faegri 1986); the North American species of *Dodecatheon* (Primulaceae; Macior 1964, 1970; Harder and Barclay 1994), and the Australian genus *Dianella* (Lilaceae; Proctor et al. 1996). In fact, there are over 70 families and 500 genera that contain at least some poricidal taxa that are often associated with vibratory pollen release (Buchmann 1983). The remarkable feature about vibratory pollen collection in the Gloxinieae is that it has multiple origins and it is autapomorphic in *Phinaea pulchella*. Thus, although this pollination syndrome is common in other groups, it has not been well documented in a phylogenetic context as demonstrated here.

Convergence of Generic Characters for Phinaea pulchella and Phinaea s. s.—Based on the results presented here, morphological characters evaluated for the recent recircumscription of *Phinaea* (Boggan et al. 2008) must be considered convergent. The following diagnostic characters for *Phinaea* outlined in the circumscription and Table 1 from Boggan et al. (2008) are convergent: erect pedicels in fruit (in contrast to curved in fruit); fruit valves fleshy at dehiscence and opening broadly (in contrast to valves dry at dehiscence and opening slightly); sticky seeds adhering to the valves (in contrast to seeds falling freely); and the presence of an annular nectary (in contrast to nectary absent). Most of these characters are related to fruit dehiscence and not flower characters (cf., discussion on lack of nectar). Flower characters are often associated with pollination syndromes and are therefore labile whereas fruit characters have been considered to be more conservative in an evolutionary context. Nevertheless, some of these characters, such as the difference between erect pedicels (e.g. *Niphaea* and *Phinaea*) and curved pedicels (e.g. *Amalophyllon*), are difficult to evaluate from herbarium collections. It is likely that even though pedicel posture appears to be a qualitative character, there may be some variability. This is difficult to evaluate without the investigation of living specimens, which were available to Boggan et al. (2008) only to a limited extent.

One of the surprising convergences outlined in Boggan et al. (2008) is the presence of an annular nectary in all three species of *Phinaea* and lack thereof in *Amalophyllon* and *Niphaea*. There are no known pollination studies of any species in *Phinaea, Amalophyllon,* or *Niphaea*, but the flower morphology suggests that vibratory pollen release is the mechanism for pollination. Thus, if the reward for the pollinator is pollen (i.e. not nectar) then there is little use for the production of nectar. The presence of a nectary in *Phinaea* is probably vestigial because the flower morphology suggests that the flowers

are buzz-pollinated. An important observation noted by Boggan el al. (2008: p. 171) was that the nectary in the two available live collections of *Phinaea* appeared reduced and produced no nectar. There is significant variation in nectary size and shape across the Gloxinieae and this character should be re-evaluated based on the phylogenetic results presented here.

Evolutionary Transition of Bilateral to Radial Symmetry—Transitions between bilateral and radial floral symmetry are common throughout the clade Asterideae (Coen and Nugent 1994; Donoghue et al. 1998) and especially within the tribe Lamiales (Wagstaff and Olmstead 1997), which includes the Gesneriaceae. While radial symmetry is plesiomorphic and bilateral symmetry is apomorphic in the tribe Asteridae (Coen and Nugent 1994; Donoghue et al. 1998) the opposite is the case in the Lamiales; bilateral symmetry is plesiomorphic and radial symmetry is apomorphic. For example, the plesiomorphic condition for Gesneriaceae is bilateral symmetry and this is well supported based on phylogenetic analyses (Smith et al. 1997; Smith 2000; Zimmer et al. 2002; Smith et al. 2004a, b). The phylogeny presented here supports this trend as *Phinaea pulchella* represents an independent origin of radial symmetry.

Cronk and Möller (1997) explored the implications of floral symmetry and the expression of the gene *cycloidea* (*cyc*) for adaxial/abaxial asymmetry. They suggested that there could be a transition from zygomorphy to actinomorphy as a mechanism promoting a wider range of generalist pollinators in extreme habitats. For example, the actinomorphic genus *Ramonda* is on the geographical edge of the primarily tropical distribution of the Gesneriaceae. *Ramonda* grows in the Pyrenees mountains of southwest Europe and the Balkan mountains of southeast Europe where lack of specialist pollinators could be an important evolutionary advantage that has led this group towards actinomophy from its zygomorphic ancestors (Cronk and Möller 1997). The evolution of generalized pollination from specialized ancestors was recently explored by Martén-Rodríguez et al. (2010) based on phylogenetic results and extensive fieldwork on the Caribbean members in the tribe Gesnerieae. Martén-Rodríguez et al. (2010) showed that specialized hummingbird pollination was ancestral to at least four origins of generalized pollination (by bats, moths, and hummingbirds). Whether or not the evolution of radial symmetry in *Phinaea pulchella* represents specialized or generalized pollination will need to be further evaluated by field studies.

Classification of Phinaea pulchella—The phylogeny presented here makes a strong case that *Phinaea pulchella* is not related to the other two currently recognized species of *Phinaea* and should be classified in a separate genus. The other two species of *Phinaea* are strongly supported as closely related based on phylogenetic analyses of nrDNA (ITS region), cpDNA (*trnL* intron and *trnL-trnF* intergenic spacer region), and a morphological dataset of 62 characters (Roalson et al. 2005a, 2008). Smith et al. (2004a, b) had previously demonstrated the polyphyly of *Phinaea* using some of the same molecular markers. The two closely related species of *Phinaea* are widely disjunct with *P. albolineata* restricted to South America (Colombia and Brazil) and *P. multiflora* restricted to Mexico. The type species for *Phinaea* is *P. albolineata*, which mandates that the generic name be retained for the clade containing *P. albolineata* and *P. multiflora*. Leaf material for DNA sequencing of *Phinaea pulchella* was not available for the phylogenetic studies of Roalson et al. (2005a, 2008) and Smith et al. (2004a, b). The inclusion of *P. pulchella* in *Phinaea* was based on the assessment and evaluation of morphological characters (Boggan et al. 2008).

There are 18 + species with rotate corollas in *Amalophyllon, Niphaea,* and *Phinaea* (Boggan et al. 2008) and of these, only five have been evaluated in a molecular phylogeny (Smith et al. 2004a, b; Roalson et al. 2005a, 2008). Only two of the 12 + species of *Amalophyllon* and one of the three+ species of *Niphaea* were included in Roalson et al. (2005a, 2008). The number of species in these two genera is probably greater. Boggan et al. (2008) outlined two putative new species without describing them because of insufficient material as "*Niphaea* sp. A" and "*Amalophyllon* sp. A."

The polyphyly of *Phinaea* and morphological convergences presented here are compelling reasons for the reclassification of *Phinaea pulchella* as a separate genus from the other two species of *Phinaea*. We argue that this is not yet prudent given that only five species representing *Amalophyllon, Niphaea,* and *Phinaea* have been included in a molecular phylogenetic analyses (Roalson et al. 2005a, 2008). Of particular importance is the lack of taxon sampling for *Amalophyllon*. Thus, the reclassification of *Phinaea pulchella* should wait until more species from these three genera are available for phylogenetic studies.

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Appendix 1. Taxa, GenBank accession numbers, (ITS, *trnL-F*; — = sequence not obtained). Collection locality and voucher specimen information with herbarium acronym in parentheses for specimens not included in Roalson et al. (2005a).

Ingroup—*Achimenes candida* Lindl., AY047065, AY047124; *Achimenes cettoana* H. E. Moore, AY047066, AY047125; *Achimenes misera* Lindl., AY047067, AY047126; *Amalophyllon clarkii* Boggan & Skog, AY702391, AY702434; *Amalophyllon divaricatum* (Poepp.) Boggan & Skog, AY047078,

AY047137; *Diastema affine* Fritsch, AY702353, AY702397; *Diastema comiferum* (DC.) Benth. ex Walp., AY702354, AY702398; *Diastema racemiferum* Benth, AY047069, AY047128; *Diastema scabrum* (Poepp.) Benth. ex Walp., AY702356, AY702400; *Diastema vexans* H. E. Moore, AY702357, AY702401; *Eucodonia andrieuxii* (DC.) Wiehler, AY047060, AY047119; *Eucodonia verticillata* (M. Martens & Galeotti) Wiehler, AY047061, AY047120; *Gloxinella lindeniana* (Regel) Roalson & Boggan, AY702361, AY702405; *Gloxinia erinoides* (DC.) Roalson & Boggan, AY047073, AY047132; *Gloxinia perennis* (L.) Fritsch in Engl. & Prantl, AY047071, AY047130; *Gloxinia xanthophylla* (Poepp.) Roalson & Boggan, AY047074, AY047133; *Gloxiniopsis racemosa* (Benth.) Roalson & Boggan, AY702364, AY702407; *Goyazia rupicola* Taubert, AY702366, AY702409; *Heppiella ulmifolia* (Kunth) Hanst.; *Heppiella viscida* (Lindl. & Paxt.) Fritsch, AY702370, AY702413; *Kohleria affinis* (Fritsch) Roalson & Boggan, AY702351, AY702395; *Kohleria allenii* Standl. & L. O. Wms., AY702371, AY702414; *Kohleria amabilis* (Planch. & Linden) Fritsch, AY702372, AY702415; *Kohleria grandiflora* L. P. Kvist & L. E. Skog, AY702373, AY702416; *Kohleria hypertrichosa* J. L. Clark & L. E. Skog, AY702376, AY702419; *Kohleria hirsuta* (Kunth) Regel, AY702374, AY702417; *Kohleria peruviana* Fritsch, AY702375, AY702418; *Kohleria rugata* (Scheidw.) L. P. Kvist & L. E. Skog, AY047075, AY047134; *Kohleria tigridia* (Ohlend.) Roalson & Boggan, AY702352, AY702396; *Kohleria trianae* (Regel) Hanst., AY702377, AY702420; *Kohleria villosa* (Fritsch) Wiehler, AY047076, AY047135; *Kohleria warszewiczii* (Regel) Hanst., AY702379, AY702422; *Mandirola ichthyostoma* (Gardner) Seem. ex Hanst., AY702360, AY702404; *Mandirola multiflora* (Gardner) Decne., AY702363, —; *Monopyle flava* L. E. Skog, AY702381, AY702424; *Monopyle puberula* C. V. Morton, AY047070, AY0471290; *Moussonia deppeana* (Schlechtend. & Cham.) Hanst., AY702383, AY702426; *Moussonia elegans* Decne., AY702384, AY702427; *Moussonia septentrionalis* (Denham) Wiehler, AY047068, AY047127; *Niphaea oblonga* Lindl., AY047064, AY047123; *Nomopyle dodsonii* (Wiehler) Roalson & Boggan, AY702358, AY702402; *Pearcea abunda* (Wiehler) L. P. Kvist & L. E. Skog, AY047077, AY047136; *Phinaea albolineata* (Hook.) Benth. ex Hemsl., AY702389, AY702432; *Phinaea multiflora* C. V. Morton, AY702390, AY702433; *Phinaea pulchella* (Griseb.) C. V. Morton, GU597365, GU597366, Cuba, *J. L. Clark et al. 10583* (US); *Pearcea hypocyrtiflora* (Hook.f.) Regel, AY702385, AY702428; *Pearcea reticulata* (Fritsch) L. P. Kvist & L. E. Skog, AY702386, AY702429; *Pearcea sprucei* (Britton) L. P. Kvist & L. E. Skog, AY702387, AY702430; *Seemannia gymnostoma* (Griseb.) Toursark., AY702359, AY702403; *Seemannia nematanthodes* (Kuntze) K. Schum., AY702362, AY702406; *Seemannia purpurascens* Rusby, AY047072, AY047131; *Seemannia sylvatica* (Kunth) Hanst., AY702365, AY702408; *Smithiantha aurantiaca* Wiehler, AY047063, AY047122; *Smithiantha canarina* Wiehler, AY047062, AY047121; *Solenophora calycosa* J. D. Sm., AY702392, AY702435; *Solenophora tuerkheimiana* J. D. Sm., AY702393, AY702436.

Outgroups—*Bellonia spinosa* Swartz, AY702350, AY702394; *Gesneria acaulis* L., AY047045, AY047104; *Gesneria pedunculosa* (DC.) Fritsch, AY047052, AY047111; *Pheidonocarpa corymbosa* (Swartz) L. E. Skog, AY702388, AY702431; *Rhytidophyllum auriculatum* Hook., AY047058, AY047117; *Rhytidophyllum exsertum* Griseb., AY047055, AY047114.