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Phylogenetic Relationships and the Diversification of Floral Form in *Achimenes* (Gesneriaceae)

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ABSTRACT. *Achimenes* is a genus in the Gesneriaceae, subfamily Gesnerioideae, tribe Gloxinieae that shows remarkable variation in floral form and possibly floral pollination syndrome. This includes flowers that are salverform, tubular, or infundibuliform, white, yellow, pink, purple, and red, and with or without corolla spurs. Previous classifications of *Achimenes* have relied heavily on floral form as a measure of relationship. This study explores phylogenetic relationships in *Achimenes* and addresses questions of proper supraspecific classification, evolution of floral forms and pollination syndromes, the origins of floral spurs, chromosome evolution, and biogeographic patterns using nrDNA ITS and cpDNA *trnL-F* spacer sequences analyzed using maximum parsimony and maximum likelihood methodologies. Phylogenetic hypotheses support the non-monophyly of most of the supraspecific classification units currently recognized in *Achimenes*, multiple origins of each of the pollination syndromes and the major morphological characteristics used to define these syndromes, multiple origins of floral spurs, multiple tetraploid events, and sympatric distributions of many closely related species.

Innovation in floral form has been proposed as one of the primary mechanisms of diversification in angiosperms (Barrett et al. 1996). This phenomenon has been most intensively studied in groups such as the Orchidaceae (Dodson 1962; Johnson et al. 1998) and the Polemoniaceae (Grant and Grant 1965), among others. The past ten years have seen an increase in the exploration of comparative floral diversity in a phylogenetic context (Armbruster 1992, 1993; Manning and Linder 1992; McDade 1992; Luckow and Hopkins 1995; Brunneau 1997; Hodges 1997; Donoghue et al. 1998; Reeves and Olmstead 1998). The combination of comparative floral morphology and phylogenetic hypotheses allows for the exploration of the kinds and number of transitions among different morphologies.

The Gesneriaceae Rich. & Juss. (African violet family) are a family of tropical herbs, shrubs, trees, lianas, and epiphytes renowned for their diversity of floral form (Wiehler 1983). Many morphological characteristics of the flowers have likely evolved multiple times in different lineages of the family (e.g., floral spurs in *Achimenes*, *Besleria* L., *Codonanthe* (Mart.) Hanst., *Drymonia* Mart., *Gasteranthus* Benth., and *Paradrymonia* Hanst.; Ramírez Roa 1987; Feuillet and Steyermark 1999; Skog and Kvist 2000). This diversification has created special difficulty in accurate classification of these genera, as flower characters are often used in their circumscription. For example, *Achimenes antirrhina* includes in its synonymy epithets in the genera *Alsobia* Hanst., *Antirrhinum* L., *Dicyrta* Regel, *Gloxinia* L'Heritier, *Guthnickia* Regel, and *Trevirana* Willd. Phylogenetic relationships in the Gesneriaceae are beginning to be understood for several lineages (Boggan 1991; Smith and Sytsma 1994; Smith 1996; Möller and Cronk 1997a, b; Samuel et al. 1997; Smith and Carroll 1997; Smith et al. 1997; Smith and Atkinson 1998; Harrison et al. 1999; Möller et al. 1999; Denduangboripant and Cronk 2000; Smith 2000a, b, c; Atkins et al. 2001; Möller and Cronk 2001; Smith 2001; Zimmer et al. 2002). These phylogenetic hypotheses of relationships are beginning to give us a better understanding of morphological evolution in the Gesneriaceae. For example, Möller and Cronk (2001) studied growth form in *Streptocarpus* Lindl. and provided evidence for the multiple origins of the caulescent, unifoliate, and rosulate growth forms in this genus.

The genus *Achimenes* Pers. is a member of the Gesneriaceae, subfamily Gesnerioideae, tribe Gloxinieae Fritsch and is distributed from northern Mexico and the Caribbean Islands south to northern South America, with a center of distribution in central and southern Mexico (Wiehler 1976; Ramírez Roa 1987). The current circumscription of *Achimenes* recognizes 22 species (Ramírez Roa 1987; Wiehler 1992). Additionally there is one recently described entity (*A. hintoniana*; Ramírez Roa and Skog 2002), and one species with two varieties (*A. flava* var. *flava* and *A. flava* var. *saxicola* (T.S. Brandegee) Ramírez Roa, ined.).

Achimenes Pers. (1806) is conserved (Dandy 1969; McVaugh 1970; Stafleu et al. 1972) over the earliest publication of this name in 1756 by P. Browne which refers to *Columnea* L. The circumscription of *Achimenes* has varied considerably since its description (Wiehler 1976; Ramírez Roa 1987). For most of the last two hundred years, *Achimenes* included species now regarded as members of *Eucodonia* Hanst., *Gloxinia*, and *Goyazia*

TABLE 1. Current classification of *Achimenes* as arranged by Fritsch (1893–1894), with additions and modifications by Moore (1960), Morton (1962), Cooke and Lee (1966), Phillips (1970), Wiehler (1976), Skog (1987), Ramírez Roa (1987), Wiehler (1995), and Ramírez Roa and Skog (2002). Species marked by an asterisk are species described after the most recent classification by Wiehler (1976) and are placed in the sections of their closest relatives as inferred from morphology.

Section *Achimenes*: **A. admirabilis* Wiehler, *A. cettoana* H.E.Moore, *A. erecta* (Lam.) H.P.Fuchs, *A. grandiflora* (Schiede) DC., *A. longiflora* DC., **A. nayaritensis* L.E.Skog, *A. patens* Benth.
Section *Dicyrta* (Regel) Fritsch: *A. brevifolia* C.V.Morton, *A. candida* Lindl., *A. fimbriata* Rose ex C.V.Morton, *A. flava* C.V.Morton var. *flava*, *A. flava* var. *saxicola* (Brandegee) Ramírez Roa, ined., *A. misera* Lindl., *A. obscura* C.V.Morton, *A. occidentalis* C.V.Morton, *A. woodii* C.V.Morton
Section *Guthnickia* (Regel) Fritsch: *A. antirrhina* (DC.) C.V.Morton
Section *Locheria* (Regel) Benth.: *A. heterophylla* (Mart.) DC., *A. pedunculata* Benth., *A. skinneri* Lindl.
Section *Plectopoma* (Hanst.) Fritsch: *A. glabrata* (Zucc.) Fritsch
Section *Scheeria* (Seem.) Fritsch: *A. dulcis* C.V.Morton, **A. hintoniana* Ramírez Roa & L.E.Skog, *A. mexicana* (Seem.) Benth. & Hook.f. ex Fritsch

Taubert (Wiehler 1976). The most complete treatment of *Achimenes* sensu lato was by Hanstein (1854, 1856). He circumscribed this group to include 23 species in eight genera, later reclassified into three genera (1865). Bentham (1876) included two genera, while Fritsch (1893–94) united these groups into *Achimenes*, recognizing eight sections, with the later addition of two monotypic sections (Fritsch 1897, 1913). In the twentieth century, numerous publications followed in which new species were described and numerous hybridizations among species were made, although without changes to the classification of the genus (Moore 1960; Morton 1962a, b; Cooke and Lee 1966).

In 1976, Wiehler removed what he considered to be discordant elements of *Achimenes*, placing them in the genera *Eucodonia*, *Gloxinia*, and *Goyazia*. This reorganization of *Achimenes* resulted in the recognition of 21 species in six sections (Wiehler 1976). Ramírez Roa (1987) revised *Achimenes* sensu stricto without major changes to the sectional circumscription of the genus. Additionally, new species continue to be described (Skog 1987; Wiehler 1992; Ramírez Roa and Skog 2002).

Achimenes sensu stricto includes a diversity of floral forms (Wiehler 1976; Ramírez Roa 1987). These floral forms can be grouped into ornithophilous, psychophilous, gynandro-euglossophilous, and melittophilous pollination syndromes (Wiehler 1976; Ramírez Roa 1987). The description of pollinator types in *Achimenes* is largely based on extrapolation from the floral biology of other groups of Gesneriaceae, not on actual observations of pollinators visiting flowers (Ramírez Roa 1987).

The ornithophilous pollination syndrome includes species with red, tubular or tubular-salverform corollas presumably pollinated by hummingbirds (Ramírez Roa 1987). Six *Achimenes* species display this syndrome: *A. admirabilis*, *A. antirrhina*, *A. erecta*, *A. heterophylla*, *A. pedunculata*, and *A. skinneri* (Wiehler 1976; Ramírez Roa 1987; Wiehler 1995). Psychophilous species are those species with purple or pink salverform corollas pollinated by butterflies (Wiehler 1976; Ramírez Roa 1987). Five species display this syndrome: *A. cettoana*, *A. grandiflora*, *A. longiflora*, *A. nayaritensis*, and *A. patens* (Wiehler 1976; Skog 1987; Ramírez Roa 1987). Species with the gynandro-euglossophilous pollination syndrome are presumably pollinated by female euglossine bees and have white or purple infundibuliform (funnel-shaped) flowers (Wiehler 1976; Ramírez Roa 1987). Four species have this syndrome: *A. dulcis*, *A. glabrata*, *A. hintoniana*, and *A. mexicana* (Wiehler 1976; Ramírez Roa 1987; Ramírez Roa and Skog 2002). The mellitophilous pollination syndrome includes species with white or yellow short-tubular flowers, often with purple or red spots marking the floral tube and presumably pollinated by bees (Wiehler 1976; Ramírez Roa 1987). Eight species exhibit this syndrome: *A. brevifolia*, *A. candida*, *A. fimbriata*, *A. flava*, *A. misera*, *A. obscura*, *A. occidentalis*, and *A. woodii* (Wiehler 1976; Ramírez Roa 1987).

The species of *Achimenes* also vary in the presence, shape, and size of a corolla floral spur (Ramírez Roa 1987). Floral spurs have often been associated with nectaries and selection for specific pollinators (Hodges 1997). However, floral spurs in *Achimenes* are not directly associated with nectary tissue, and their function is unclear. Many species of *Achimenes* lack a spur altogether (11 species), others have various degrees of a gibbous corolla base (6 species), and some have a well-developed spur up to 1 cm long (6 species).

The most recent classification of *Achimenes* recognizes 23 species in six sections: *Achimenes*, *Dicyrta*, *Guthnickia*, *Locheria*, *Plectopoma*, and *Scheeria* (Wiehler 1976; Ramírez Roa 1987; Table 1). These sections are primarily based on pollination syndromes associated with these groups of species (Wiehler 1976; Ramírez Roa 1987).

Recent phylogenetic studies of the Gesnerioideae strongly support *Achimenes* as a member of the Gloxinieae sensu stricto, excluding *Sinningia* Nees and relatives and *Gloxinia sarmentiana* Gardner ex Hook. (Zimmer et al. 2002). Species removed by Wiehler (1976)

TABLE 2. Voucher information and GenBank accession numbers for species of *Achimenes* and *Moussonia* included in the analyses. All vouchers are deposited at US. Samples taken from live material growing at the U.S. Botany Research Greenhouses (USBRG) are designated by their accession number and a voucher collection, when present. Seed collections received from the American Gloxinia and Gesneriad Society seed fund are abbreviated AGGS SF. Species where more than one collection was sampled are numbered in parentheses for cross reference with their individual placement on the phylogenetic trees.

Moussonia septentrionalis: USBRG 99-001, *Skog 8045*; ITS AY047068, *trnL-F* AY047127. ***M. deppeana***: AGGS SF, *Skog 8231*; ITS AY182172, *trnL-F* AY182201.

Achimenes admirabilis: USBRG 94-553, *Skog 8196*; ITS AY182173, *trnL-F* AY182202. ***A. antirrhina***: *Lott 3010*; ITS AY182190, *trnL-F* AY182219. ***A. candida*** (1): USBRG 96-150, *Skog 7840*; ITS AY047065, *trnL-F* AY047124. ***A. candida*** (2): USBRG 00-201, *Skog 8265*; ITS AY182174, *trnL-F* AY182203. ***A. cettoana***: USBRG 94-235, *Skog 7991*; ITS AY047066, *trnL-F* AY047125. ***A. dulcis*** (1): USBRG 94-113, *Skog 7772*; ITS AY182175, *trnL-F* AY182204. ***A. dulcis*** (2): USBRG 00-203, Skog 8266; ITS AY182176, *trnL-F* AY182205. ***A. erecta*** (1): *Skog 7022*; ITS AY182189, *trnL-F* AY182218. ***A. erecta*** (2): USBRG 00-199, *Skog 8226*; ITS AY182188, *trnL-F* AY182217. ***A. fimbriata***: *Sanders 21035*; ITS AY182193, *trnL-F* AY182222. ***A. flava***: USBRG 94-122, *Skog 7957*; ITS AY182177, *trnL-F* AY182206. ***A. glabrata***: *Anderson 12845*; ITS AY182194, *trnL-F* AY182223. ***A. grandiflora*** (1): USBRG 94-323, *Skog 8162*; ITS AY182178, *trnL-F* AY182207. ***A. grandiflora*** (2): *Skog and Kopp 7565*; ITS AY182179, *trnL-F* AY182208. ***A. heterophylla***: USBRG 00-238, *Skog 8229*; ITS AY182199, *trnL-F* AY182228. ***A. hintoniana***: *McVaugh et al. 16336*; ITS AY182197, *trnL-F* AY182226. ***A. longiflora*** (1): USBRG 00-197, *Skog 8267*; ITS AY182180, *trnL-F* AY182209. ***A. longiflora*** (2): USBRG 00-198, *Skog 8268*; ITS AY182181, *trnL-F* AY182210. ***A. mexicana*** (1): USBRG 94-131, *Skog 7949*; ITS AY182186, *trnL-F* AY182215. ***A. mexicana*** (2): USBRG 94-476, *Skog 8269*; ITS AY182187, *trnL-F* AY182216. ***A. misera*** (1): *Skog 7667*; ITS AY182185, *trnL-F* AY182214. ***A. misera*** (2): USBRG 00-195, *Skog 8222*; ITS AY182184, *trnL-F* AY182213. ***A. nayaritensis***: *McVaugh 18985*; ITS AY182196, *trnL-F* AY182225. ***A. occidentalis***: *McVaugh 17346*; ITS AY182200, *trnL-F* AY182229. ***A. patens*** (1): USBRG 94-120, *Skog 8014*; ITS AY182182, *trnL-F* AY182211. ***A. patens*** (2): USBRG 95-061, *Skog 7946*; ITS AY182183, *trnL-F* AY182212. ***A. pedunculata*** (1): *Glicenstein s.n.*; ITS AY182192, *trnL-F* AY182221. ***A. pedunculata*** (2): UBBRG 00-244, *Skog 8026*; ITS AY182198, *trnL-F* AY182227. ***A. pedunculata*** (3): USBRG 98-115, *Skog 7849*; ITS AY182191, *trnL-F* AY182220. ***A. woodii***: *Rzedowski 35745*; ITS AY182195, trnL-F AY182224.

from *Achimenes* belong to the genera *Eucodonia*, *Gloxinia*, and *Goyazia* (Wiehler 1976, 1983). Previous studies have supported the separation of some of the species (e.g., those now in *Eucodonia*) from *Achimenes* (Zimmer et al. 2002). Species of *Gloxinia* and *Goyazia* once considered as part of *Achimenes* appear to be associated with lineages separate from *Achimenes* (E. H. Roalson, unpubl. data). Additionally, *Achimenes* does not appear to be closely related to any of other genera in the Gloxinieae, and may represent the sister lineage to the rest of the Gloxinieae (Zimmer et al. 2002). This study explores phylogenetic relationships within *Achimenes* using the nrDNA internal transcribed spacer region (ITS) and the cpDNA *trnL* intron, and *trnL-F* intergenic spacer regions. Specifically, we address: (1) the phylogenetic patterns of change in flower phenotype associated with different pollination syndromes; (2) the evolutionary origin of floral spurs in *Achimenes*; (3) evolutionary origins of other morphological characters; and (4) the classification of *Achimenes* in light of the phylogenetic hypotheses.

MATERIALS AND METHODS

Sampling. Samples were selected from live plants grown at the Smithsonian National Museum of Natural History Botany Research Greenhouses and herbarium specimens at US. Outgroup sampling included two samples of the genus *Moussonia* which was supported in Zimmer et al. (2002) as a distinct lineage from *Achimenes* and likely one of the nearest relatives of the genus (Table 2). Previous studies have strongly supported the monophyly of the Gloxinieae s.s. and weakly supported the monophyly of *Achimenes* (Zimmer et al. 2002; E. H. Roalson, unpubl. data). The ingroup comprised 30 samples of 20 species representing all currently recognized species of *Achimenes* except *A. brevifolia*, *A. obscura*, and *A. skinneri* (Table 2). Sequencing of *Achimenes brevifolia* and *A. obscura* samples was attempted but only partial sequences of the cpDNA spacers was successful. Since only a small portion of the variable sites were sequenced, these species were excluded from the main analyses, but their phylogenetic position is explored in the discussion. All of the samples of *Achimenes skinneri* available appear to be hybrids. This conclusion is based on morphologically polymorphic individuals from seed sources as well as sequences that were quite polymorphic, and for this reason, these sequences were excluded from the analyses.

Morphological characters mapped onto the phylogenetic trees are based on examination of live and herbarium specimens as well as reports from the literature (Morton 1962b; Ramírez Roa 1987; Skog 1987; Wiehler 1995).

DNA Sequencing. DNA was isolated using the Quiagen DNeasy™ DNA isolation kit. Templates of the nrDNA internal transcribed spacer region (ITS) were prepared using the primers ITS5HP (5′-GGA AGG AGA AGT CGT AAC AAG G-3′; Suh et al. 1993) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′; White et al. 1990). The chloroplast spacer regions were amplified using the primers trnLc (5′-CGA AAT CGG TAG ACG CTA CG-3′) and trnLf (5′-ATT TGA ACT GGT GAC ACG AG-3′) for the *trnL* intron and *trnL-trnF* intergenic spacer (igs; Taberlet et 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 ITS5 and ITS2 (5′-GCT GCGTTC TTC ATC GAT GC-3′), the ITS2 spacer was amplified with ITS4 and ITS3 (5′-GCA TCG ATG AAG AAC GCA GC-3′), the *trnL* intron was amplified with trnLc and trnLd (5′-GGG GAT AGA GGG ACT TGA AC-3′), and the *trnL-F* igs was amplified with trnLf and trnLe (5′-GGT TCA AGT CCC TCT ATC CC-3′). Polymerase chain-reaction (PCR) amplifications followed standard procedures described by Zimmer et 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 and Soltis 1995).

Sequencing was performed using an Applied Biosystems Model 377 Automated DNA Sequencing System. Direct cycle-sequencing

of purified template DNAs followed the manufacturer's specifications for the ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems). Sequencing of the ITS region utilized the primers ITS5HP and ITS4, or ITS5 and ITS2 for the ITS1 spacer and ITS4 and ITS3 for the ITS2 spacer. Sequencing of the chloroplast spacers made use of trnLc and trnLf, or trnLc and trnLd for the *trnL* intron and trnLe and trnLf primers for the *trnL-F* igs.

Automated DNA sequencing chromatograms were proofed, edited, and contigs were assembled using Sequencher 3.0 (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 et al. 2002). All sequences were manually aligned.

Amplification of DNA from some *Achimenes* species posed particular difficulty. A few *Achimenes* species have been rarely collected or are known from a single collection (e.g., *A. brevifolia*). Amplification of DNA from herbarium material is common in phylogenetic studies, but is a difficult matter in many Gesneriaceae (E. H. Roalson, unpubl. data). For these reasons, some species of *Achimenes* are represented by only partial sequences. Where large portions of the sequences are missing, the specimens were excluded from the primary analyses and their relationship with other *Achimenes* species is discussed (see Discussion).

All sequences were deposited in GenBank (accessions AY182172 to AY182229; Table 2).

Phylogenetic Analyses. Maximum parsimony (MP) analysis was performed using PAUP* 4.0b6 (Swofford 2001). The analysis used heuristic searches (ACCTRAN; 100 random addition cycles; TBR branch swapping; STEEPEST DESCENT; ''gap'' states treated as missing). The MP analysis of cpDNA spacers was limited to 500 trees of equal length for each of the 100 replicates due to the number of equal length trees. Clade robustness was estimated using the 100 heuristic bootstrap replicates (10 random addition cycles with 100 trees saved per cyle, TBR banch swapping; STEEPEST DESCENT; Felsenstein 1985; Hillis and Bull 1993). Three ingroup data sets were analyzed and compared: ITS, *trnL-F,* and a combination of these. For each data set, a number of insertion deletion events (indels) were coded as binary characters and included in the analysis of their respective data sets and the combined data analyses. Autapomorphic and complex gaps were not included. The data set is available on TreeBASE (study accession number = S915; matrix accession numbers = M1514 and M1515).

Homogeneity of the ITS and *trnL-F* data sets was assessed using the partition homogeneity test (Farris et al. 1995) as implemented in PAUP*4.0b6. 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 data sets and trees derived from the defined data partition.

The nrDNA ITS spacer regions were additionally analyzed with maximum likelihood (ML) as implimented in PAUP*4.0b6 (Swofford 2001). Heuristic searches were employed (ACCTRAN; starting tree based on neighbor-joining reconstruction; TBR branch swapping). The TrN (Tamura and Nei 1993) model of evolution with an estimated gamma shape parameter and estimated proportion of invariant sites was used in the ML analysis of ITS based on the results of analyses using Modeltest 3.0 (Posada and Crandall 1998). The Modeltest 3.0 analysis tests the fit of various ML models to the data set and estimates base change frequencies, proportion of variable characters, and shape of the gamma distribution, and chooses the model that best fits the data using the Hierarchical Likelihood Ratio Test (Posada and Crandall 1998). The parameters assigned to the ITS data set for this analysis were as follows: estimated base frequencies (A = 0.2110, C = 0.2781, G = 0.2618, T = 0.2491), three substitution types, proportion of sites assumed to be invariable = 0, rates for variable sites assumed to follow a gamma distribution with shape parameter = 0.3555, and a substitution rate matrix of A/C: 1.0000, A/G: 2.4675, A/T: 1.0000, C/G: 1.0000, C/T: 5.1441, and G/T: 1.0000. The *trnL-F* data set was not analyzed with ML due to the very few informative characters and minimal resolution with MP analyses (see results below).

RESULTS

DNA Sequencing and Alignment. 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 649 bp long with 172 variable sites, of which 114 were parsimony informative, plus 12 coded indels. The length of the unaligned sequences varied from 597 to 627 bp. One sequence is missing a portion (25 aligned bp) within the ITS1 spacer due to poor sequencing of that region. The alignment resulted in 21 gaps ranging from 1 to 32 bp in length. Eight of these gaps were single base indels. This data alignment resulted in uncorrected pairwise sequence divergence within the ingroup of 0% to 11%.

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 907 bp long with 36 variable sites, of which 19 were parsimony informative, plus eight coded indels. The length of the unaligned sequences (excluding those samples missing the entire intron or igs) varied from 883 to 899 bp for the *trnL-F* spacer region. Twenty sequences are missing a portion (6–123 aligned bp) of the 5′ end of the *trnL* intron, and one sequence is missing 12 aligned bp of the *trnL* exon 2, due to poor sequencing of these regions. One sample is missing the entire *trnL* intron and a portion of the *trnL* exon 2 (*A. erecta* [1]; 536 aligned bp) and three samples are missing a portion of the *trnL* exon 2 and the entire *trnL-F* igs (*A. antirrhina*, *A. pedunculata* [1], and *A. glabrata*; 363 aligned bp). The alignment resulted in 13 gaps ranging from 1 to 10 bp in length. Eight of these gaps were single base indels. This data alignment resulted in uncorrected pairwise sequence divergence within the ingroup of 0% to 1.6%.

Maximum Parsimony Analyses. Maximum parsimony analysis of the ITS *Achimenes* data set resulted in 308 most-parsimonious trees (length = 293 steps, CI = 0.744, RI = 0.864, RC = 0.643). Figure 1a is the strict consensus of these trees. Maximum parsimony analysis of the *trnL-F Achimenes* data set resulted in 10,000 most-parsimonious trees (length = 48 steps, CI = 0.917, RI = 0.949, RC = 0.870). Figure 1b is the strict consensus of these trees. Maximum parsimony analysis of the combined ITS/*trnL-F Achimenes* data set resulted in 10 most-parsimonious trees (length = 338 steps, CI = 0.763, RI = 0.871, RC = 0.665). Figure 2 is the strict consensus of these trees.

Tests of Conflict Between Data Sets. The partition

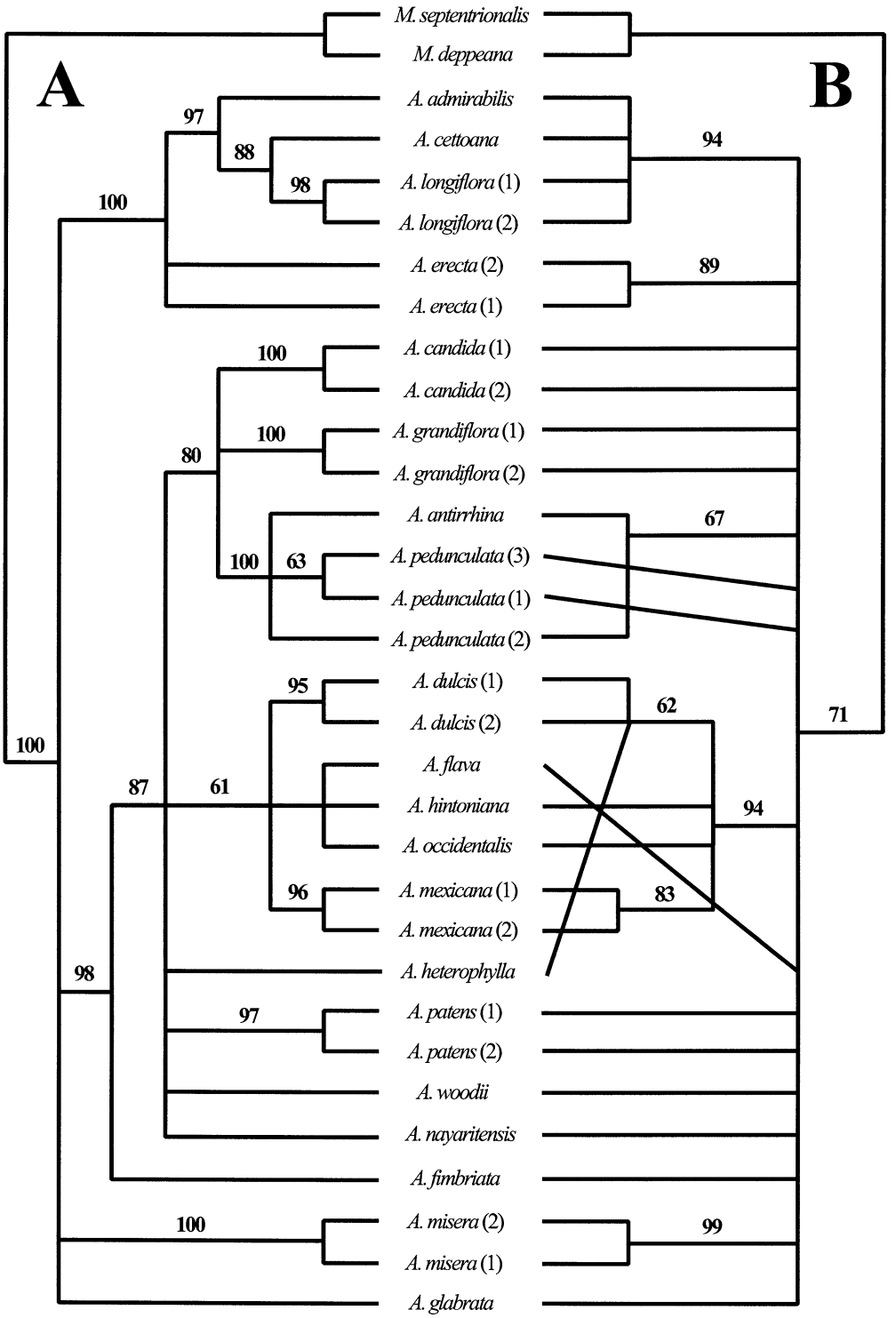

FIG. 1. Analysis of relationships within *Achimenes*. A. nrDNA ITS data set strict (MP) consensus tree of 308 most-parsimonious trees of 293 steps (CI = 0.744, RI = 0.864, RC = 0.643). Numbers above branches are bootstrap percentages where branch support is greater than 50%. B. cpDNA *trnL-F* data set strict (MP) consensus tree of 10,000 most-parsimonious trees of 48 steps (CI = 0.917, RI = 0.949, RC = 0.870). Numbers above branches are bootstrap percentages where branch support is greater than 50%. Genera are abbreviated as follows: *A.* = *Achimenes* and *M.* = *Moussonia*.

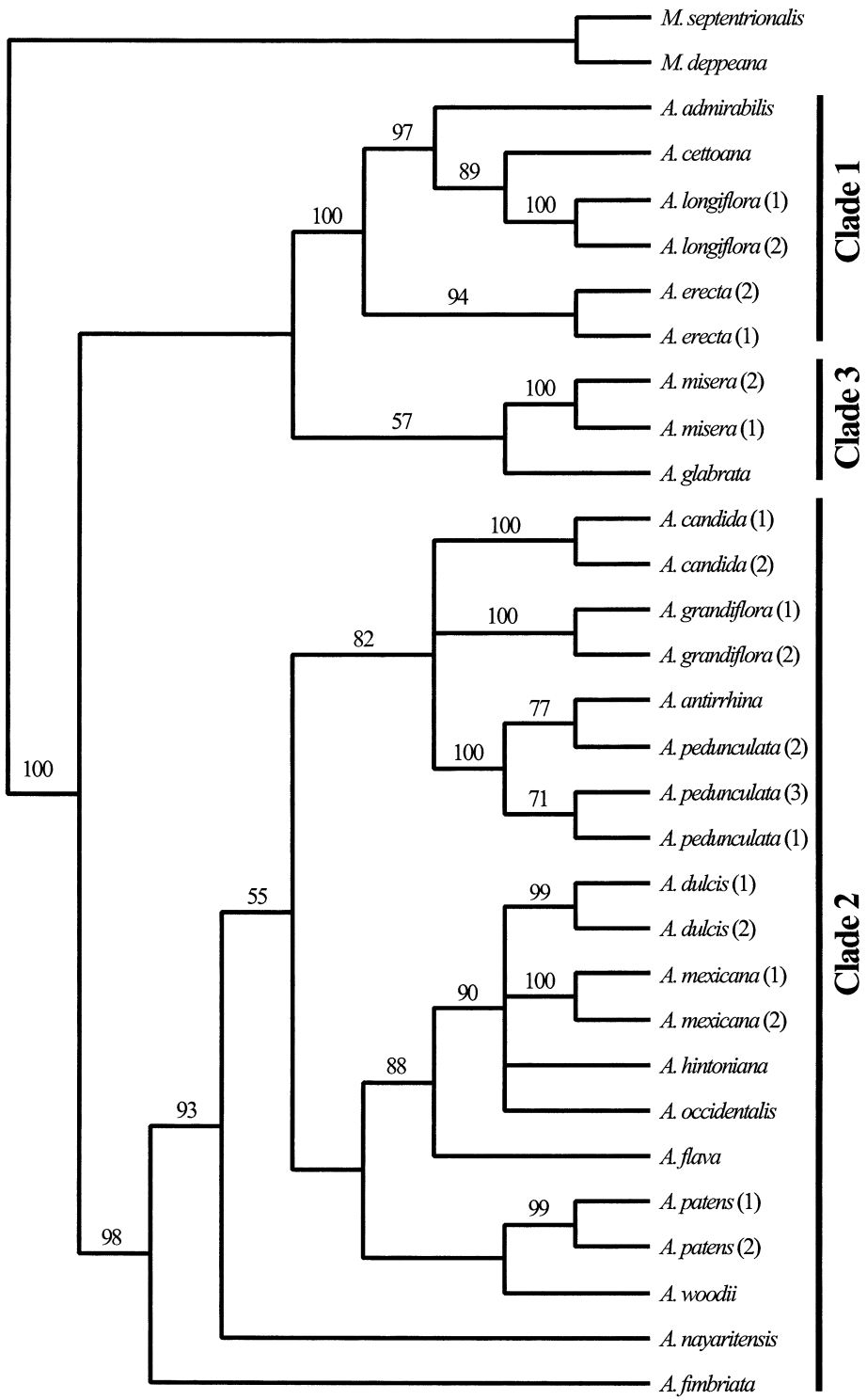

FIG. 2. Analysis of relationships within *Achimenes* using the combined nrDNA ITS/cpDNA *trnL-F* data set. Strict (MP) consensus tree of 10 most-parsimonious trees of 338 steps (CI = 0.763, RI = 0.871, RC = 0.665). Numbers above branches are bootstrap percentages where branch support is greater than 50%. Genera are abbreviated as follows: *A.* = *Achimenes* and *M.* = *Moussonia*.

homogeneity test found a significant difference between the ITS/*trnL-F* partition and random partitioning (P=0.046000). Generally, the ITS and *trnL-F* individual analyses are congruent, with some slight differences among poorly supported nodes (Figs. 1a and 1b). The primary difference between the two analyses is the placement of *A. heterophylla* and, to a lesser degree, *A. flava*. When *A. heterophylla* is excluded from the PHT analyses, the result is a non-significant difference between the ITS/*trnL-F* partition and random partition (P=0.865100). For this reason, *A. heterophylla* is excluded from the combined data MP analysis, as the incongruence between the ITS and *trnL-F* data sets implies our sample of *A. heterophylla* may be of hybrid origin.

The combined data parsimony analysis trees combine the aspects of the ITS and *trnL-F* topologies (Figs. 1–2). Strongly supported nodes based on only one data set are present and strongly supported in the combined analysis (e.g., the *A. erecta/A. admirabilis/A. cettoana/A. longiflora* clade; Figs. 1–2).

Maximum Likelihood Analysis. The ML analysis of the ITS data set examined 27,040 rearrangements. One tree (-ln = 2440.97335) was found (Fig. 3). The ITS ML analysis results in a topology that is mostly congruent with the combined data MP topology. The placement of two taxa differ between the topologies: *A. flava* is placed in a clade with *A. hintoniana* and *A. occidentalis* in the ITS ML tree whereas it is placed as sister to the *A. dulcis/A. hintoniana/A. mexicana/A. occidentalis* clade in the combined data MP consensus tree, and *A. woodii* is placed sister to the *A. dulcis/A. flava/A. hintoniana/A. mexicana/A. occidentalis/A. patens* clade in the ITS ML tree but placed as sister to *A. patens* in the combined data MP consensus tree (Figs. 2 and 3).

Discussion

Comparison of ITS, trnL-F, and Combined Data Maximum Parsimony and Maximum Likelihood Analyses. The ITS MP analysis provides a largely resolved phylogeny of *Achimenes*. Two major clades are stongly supported (the *A. admirabilis/A. cettoana/A. erecta/A. longiflora* clade [bs = 100%] and the *A. antirrhina/A. candida/A. dulcis/A. fimbriata/A. flava/A. grandiflora/A. heterophylla/A. hintoniana/A. mexicana/A. nayaritensis/A. occidentalis/A. patens/A. pedunculata/A. woodii* clade [bs = 98%]), with two taxa (*A. glabrata* and *A. misera*) remaining unresolved in relation to these two clades (Fig. 1a). All species represented by multiple samples are supported as monophyletic except *A. pedunculata* which is unresolved in relation to *A. antirrhina*, and *A. erecta* which is unresolved in relation to the *A. admirabilis/A. cettoana/A. longiflora* clade. Several additional clades are supported by moderate to high bootstrap support values (Fig. 1a).

In contrast, the *trnL-F* phylogeny is largely unresolved, with only three groups of species present in the strict consensus of most-parsimonious trees (*A. admirabilis/A. cettoana/A. longiflora*, *A. dulcis/A. heterophylla/A. hintoniana/A. mexicana/A. occidentalis*, and *A. antirrhina/A. pedunculata* [2]; Fig. 1b). This analysis included four samples which were missing up to approximately half of the *trnL-F* nucleotide sites. When these samples are excluded, there is not a significant increase in resolution in the strict consensus (data not shown), nor is there an increase in bootstrap support for the nodes present. The lack of resolution can be largely attributed to the dearth of parsimony informative characters in this data set (27 including coded indels) given the number of samples (32), and the low pairwise distances among all of the taxa (<2%).

As noted previously, there is little conflict between the ITS and *trnL-F* data sets with the exclusion of *A. heterophylla*. The combined parsimony analysis is more resolved than either the *trnL-F* or ITS analyses individually (Figs. 1–2). Two primary clades are strongly supported (clade 1: bs = 100% and clade 2: bs = 98%; Fig. 2) with a third clade weakly supported (clade 3: bs = 57%). Clade 1 includes *Achimenes admirabilis*, *A. cettoana*, *A. erecta*, and *A. longiflora* with all nodes completely resolved with strong support (bs = 89–100%; Fig. 2). Clade 2 is further resolved into two moderately supported clades (the *A. antirrhina/A. candida/A. grandiflora/A. pedunculata* clade [bs = 82%] and the *A. dulcis/A. flava/A. heterophylla/A. hintoniana/A. mexicana/A. occidentalis* clade [bs = 88%]), and a third clade with weak support (*A. patens* and *A. woodii* [bs < 50%]). This group of three clades plus *A. nayaritensis* form a well-supported clade (bs = 93%) separate from the last species of clade 2, *A. fimbriata*. Clade 3 includes *A. glabrata* and *A. misera*, but this pair is only weakly supported (bs = 57%).

Two species (*A. brevifolia* and *A. obscura*) were not included in the above analyses due to the large percentage (>50%) of missing data in the combined data matrix. Specifically, while portions of the *trnL-F* spacer sequences were obtained, no portion of the ITS spacers were successfully sequenced. The *A. brevifolia* material is from the type collection, collected in 1937 (*Hinton 10766* [US]), and it is the only known collection of this species. The *A. obscura* sample is from a more recent collection (1987; *Koch, Fryxell, and Altman 87270* [US]), but no success was found with any other material of this species. While the missing data forced the exclusion of these species from the analyses, the fragments that were sequenced give a hint of their relationship to the rest of the *Achimenes* species. Specifically, both of these species clearly share several indels in the *trnL-F* spacers with the *A. misera* samples (data not shown). While their exact affinity is equivocal, they are most likely related to *A. misera*, and were placed as unre-

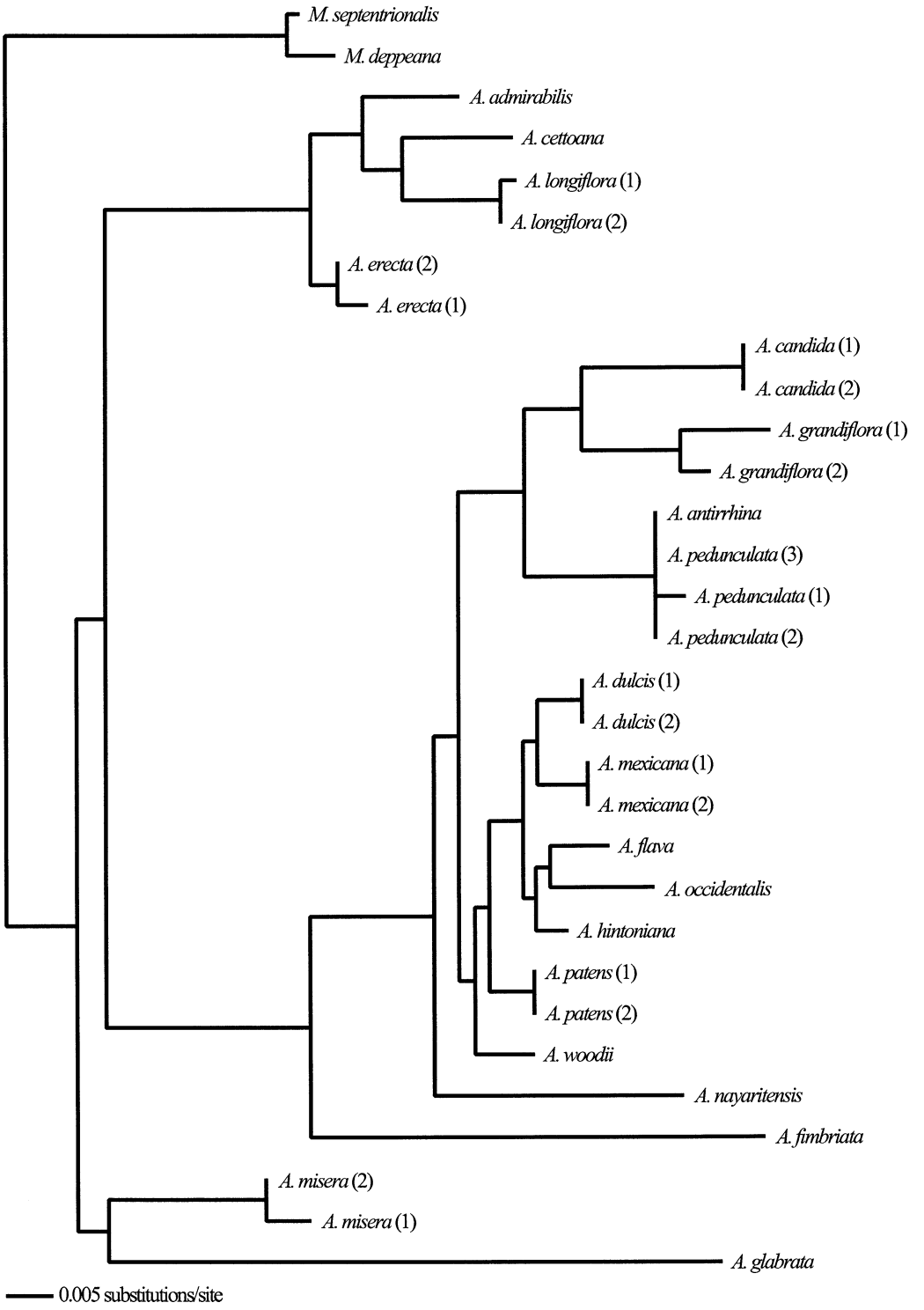

FIG. 3. *Achimenes* ITS maximum likelihood tree (-ln = 2440.97335). Genera are abbreviated as follows: *A.* = *Achimenes* and *M.* = *Moussonia*.

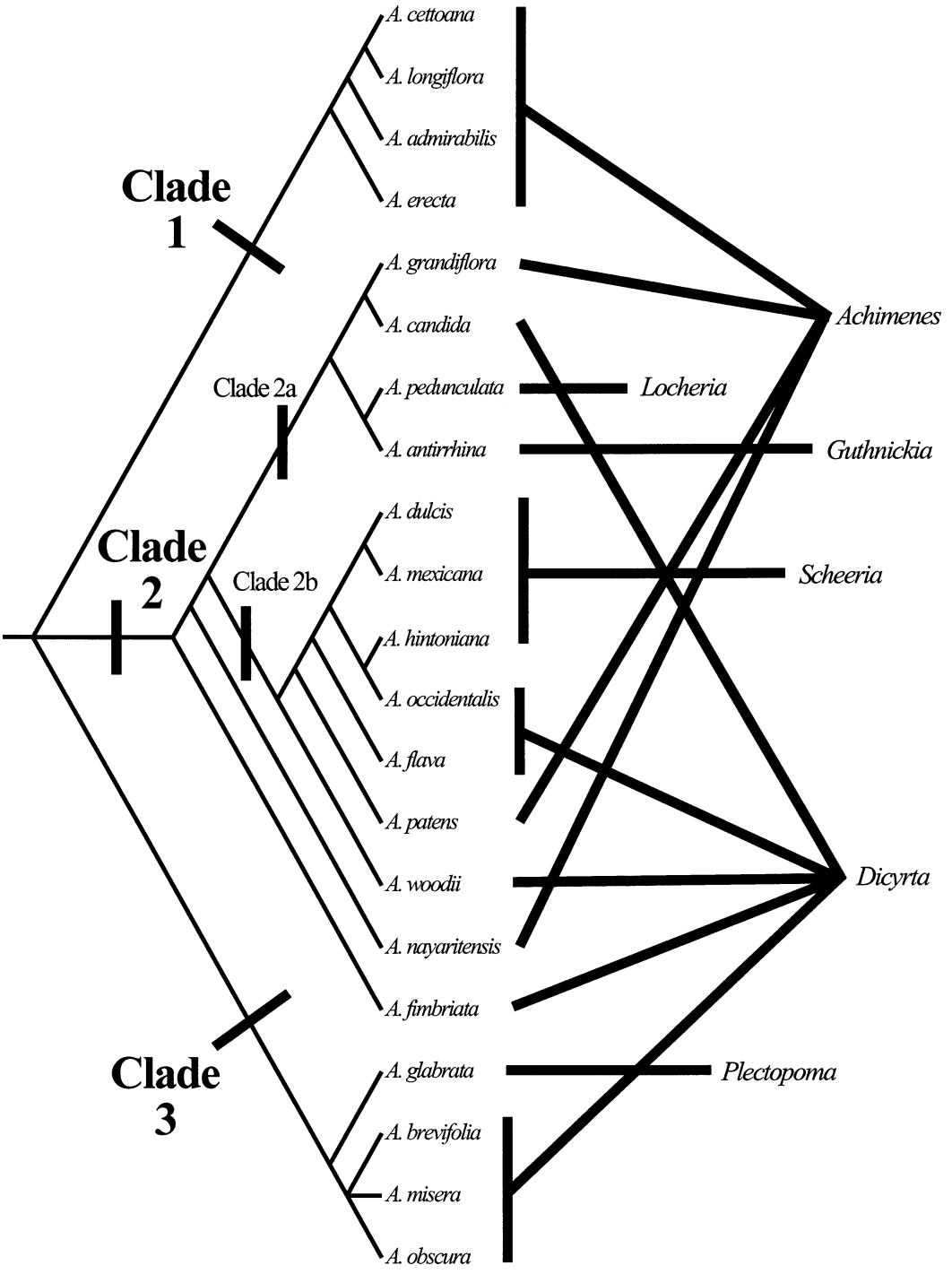

FIG. 4. Phylogenetic hypothesis of relationships in *Achimenes*. The most recent sectional classification (Wiehler, 1976) is drawn to the right of the species names. Genus abbreviation is as follows: *A.* = *Achimenes*.

solved in relation to *A. misera* in the phylogenetic hypothesis (Fig. 4).

The third species that was not included in the analysis is *A. skinneri*. All live samples of *A. skinneri* that were available appear to be of hybrid origin. This deduction is based on several lines of evidence. One of the samples explored was a seed sample from the American Gloxinia and Gesneriad Society seed fund. When these seeds were germinated, the seedlings showed a wider range of phenotypic variability than

is typical for these species. Additionally, some sequence data was gathered, and appeared to be highly polymorphic, indicating the possibility of multiple ITS types (data not shown). The small percentage of clean data gathered appeared to place *A. skinneri* with *A. longiflora*. Given the vegetative morphological support of the clade including *A. longiflora* (see discussion below) and the significantly different vegetative morphology of *A. skinneri*, the polymorphic sequence data, and the common interspecific hybridization of *A. longiflora* with other species of *Achimenes* (Becker 1996), we consider the *A. skinneri* plants in question to be hybrids. Unfortunately, amplification of the regions under study from herbarium material was unsuccessful. Given the lack of sequence data for *A. skinneri* and the fact that the other two species of section *Locheria* do not form a monophyletic group, we are unable to even tentatively place *A. skinneri* in the phylogenetic hypothesis. The phylogenetic placement of *A. skinneri* will have to wait for sequencing of verifiable collections of this species.

Similarly, the sequence data suggest that the *A. heterophylla* sample included here may be of hybrid origin. While the sequenced regions were not highly polymorphic as was found with the *A. skinneri* collections, the sequences from the nuclear and chloroplast genomes suggest significantly different placement of this species within clade 2 (Figs. 1a and 1b). Other collections of *A. heterophylla* need to be studied to determine this species true phylogenetic affinity. Reasons for the lack of congruence of nrDNA and cpDNA sequences could include lineage sorting and the presence of paralogous loci for ITS. In order to explore these possibilities, additional collections from natural populations are necessary.

The consensus phylogenetic hypothesis is presented in Figure 4. This tree is the consensus of the topologies of the combined ITS/*trnL-F* MP analysis and ITS ML analysis with the addition of *A. brevifolia* and *A. misera* based on the partial sequence fragments. This topology is used as the phylogenetic hypothesis for all subsequent discussion and the mapping of classification and morphological and cytological characters. While there has been a strong argument against using consensus trees as phylogenetic hypotheses (Carpenter 1988; Wiley et al. 1991), we feel there is good reason for using a consensus tree here. The primary rationale for our choice is that we view Fig. 4 as the best representation of all of the information on relationships and as a ''hypothesis of relationships among the species'' as opposed to merely a strict consensus of the trees. This is significant for several reasons. Primarily, if a character is mapped onto a single tree, or if only a single tree is considered in other regards, state changes are inferred on branches that are not present in all most parsimonious trees, so changes are inferred at nodes where there is no confidence in the topology. By using the ''consensus tree as a hypothesis'' rationale you avoid mapping characters onto branches you have no confidence are representing reality, and thus inferring character state changes that would likely change given more data or another tree from the same analysis. While we realize that this often creates polytomies that limit the ability to recreate character state changes, we consider this a more conservative method that minimizes inference of character state changes on branches that there is little confidence in. We include *A. brevifolia* and *A. obscura* in the phylogenetic hypothesis based on partial *trnL-F* data, but since these data do not support these species as forming a sister-pair or that one of these species is more closely related to *A. misera* than the other, the three species are placed in a tritomy. Node structure in the phylogenetic hypothesis follows the ML topology where the nodes of the combined MP consensus are unresolved or poorly supported. Where the ITS ML topology conflicts with strongly supported nodes of the combined ITS/*trnL-F* MP consensus tree, the strongly supported MP node is preferred (e.g., the placement of *A. flava*).

Clasification, Species Boundaries, and the Phylogenetic Hypothesis. The molecular phylogenetic hypothesis of relationships in *Achimenes* is not congruent with previous classifications of the genus (Fritsch 1893–1894; Wiehler 1976; Ramírez Roa 1987; Table 1; Fig. 4). Those classifications of *Achimenes* were based primarily on pollination syndromes, implicitly assuming that species with the same pollination syndrome are most closely related (Fritsch 1893–1894; Wiehler 1976). The majority of section *Achimenes* forms a clade that includes four species (clade 1, Fig. 4), but *A. grandiflora* and *A. patens* are strongly supported as members of clade 2. Additionally, *A. nayaritensis*, published after the most recent classification of *Achimenes* (Wiehler 1976), fits the morphological definition of section *Achimenes* but is a member of clade 2 according to the molecular data.

The other large section, section *Dicyrta*, is similarly polyphyletic (Fig. 4). Three of the species form a monophyletic group (clade 3, Fig. 4), but the other five are scattered throughout clade 2. The three species of *Dicyrta* in clade 3 all have infundibuliform corollas whereas only one of the five *Dicyrta* species in clade 2 has an infundibuliform corolla (*A. fimbriata*). The other four (*A. candida*, *A. flava*, *A. occidentalis*, and *A. woodii*) have salverform corollas.

The two species of section *Scheeria* (*A. dulcis* and *A. mexicana*) form a strongly supported clade with *A. hintoniana* ined. (inferred to be a member of *Scheeria*) and *A. occidentalis* of section *Dicyrta*, although the relationships of the four species are only weakly supported (Figs. 2 and 4). Section *Locheria* is represented by a single sample in the phylogenetic hypothesis (*A. pe-*

dunculata) which strongly groups with the sample of the monotypic section *Guthnickia* (*A. antirrhina*; bs = 100%). *Achimenes heterophylla* of section *Locheria* does not appear closely related to *A. pedunculata* based on either the ITS or *trnL-F* topologies, suggesting this section is likely not monophyletic. Section *Plectopoma* is monotypic (*A. glabrata*) and does not appear to be closely related to any of the other species sampled, although it is weakly supported as being part of clade 3 (Figs. 2 and 4).

There are several species-boundaries questions as yet unresolved in *Achimenes*. The most difficult of these is the separation of *A. misera* and *A. warszewicziana. Achimenes warszewicziana* has been considered part of *A. misera* (Fritsch 1893–1894; Gibson 1974), or a separate species (Moore 1962; Morton 1962b; Wiehler 1976; Ramírez Roa 1987). Recently, some authors have again questioned the recognition of *A. warszewicziana* (A. Ramírez Roa, pers. comm.). As has been noted previously (Morton 1962b), the primary characters separating *A. misera* and *A. warszewicziana* involve the number of grooves on the dorsal surface of the corolla tube and the coloration patterns on the corolla—characteristics that are difficult if not impossible to differentiate on dried herbarium specimens. While more detailed population-level studies of this complex are necessary, one species (*A. misera*) was recognized here. A number of specimens previously designated as *A. misera* and *A. warszewicziana* were included in preliminary analyses (data not shown) and all samples grouped in clade 3 with no resolution among the samples.

Where multiple samples of a species were included in the analyses, all form monophyletic groups with the exception of *A. pedunculata*. The combined data MP consensus suggests that *A. antirrhina* is nested within *A. pedunculata* whereas the ITS ML topology suggests that the two species are unresolved in relation to each other. While *A. antirrhina* and *A. pedunculata* have been placed in separate sections, *Guthnickia* and *Locheria*, respectively, they are quite similar in many regards. *Achimenes antirrhina* was placed in section *Guthnickia* based on its stomatomorphic stigmas and predominately yellow corollas whereas the three species of section *Locheria* are characterized by red to orange corollas with laterally bifurcate stigmas. While *A. antirrhina* has a tubular corolla in contrast to the tubular-infundibuliform corolla of *A. pedunculata*, *A. heterophylla* of section *Locheria* also has a tubular corolla. The possible paraphylly of *A. pedunculata* in relation to *A. antirrhina* may represent incomplete lineage sorting of the molecular markers in the separation of these two species.

As may be noted, three samples of *A. pedunculata* were included rather than one or two as in other species. This was done primarily to include *A. pedunculata* (3; Table 2). This collection is from Ecuador, significantly further south than other known localities of this species. Vegetatively, it is somewhat different from the typical *A. pedunculata* morphology, but it clearly is very similar in ITS and cpDNA type as those cultivated and natural collections from further north. Whether this collection is an escape from cultivation in Ecuador or a natural population is unclear at this time.

Evolution of Floral Form and Pollination Syndromes. Floral form appears to be quite variable among closely related species in *Achimenes* and extremely similar corolla shapes are found among species that occur in different clades (e.g., salverform corollas in *A. longiflora* and *A. grandiflora*). The three categories of flower shape, salverform, infundibuliform, and tubular, all appear to have have multiple derivations (Fig. 5). Similarly homoplastic is corolla color, with multiple derivations of white, purple, yellow, and red corollas (Fig. 5). Stigma shape (stomatomorphic, laterally bilabiate, or dorsiventrally bilabiate) has changed at least six times, and, apparently, independent of both flower shape and flower color (Fig. 5). Given the extreme diversity in these morphological characters among closely related species, the most likely explanation of this diversity is strong selection on these characters by a variety of pollinators. This pattern of difference in pollination syndrome among closely related species has also been found in other groups such as *Dalechampia* (Euphorbiaceae; Armbruster 1993), *Disa* (Orchidaceae; Johnson et al. 1998), and Polemoniaceae (Grant and Grant 1965).

Floral spurs have been inferred to be involved in pollinator specificity in some species (*Aquilegia* [Ranunculaceae]; Hodges 1997). In groups where the nectary tissue is part of the floral spur, access to nectar is regulated through changes in the length of the spur (Nilsson 1988; Johnson and Steiner 1997). In *Achimenes*, the nectary forms a ring around the base of the ovary, and is not directly associated with the spur. Additionally, the angle of flower presentation does not suggest that the spur forms a reservoir for the nectar to gather, as is found in some species of *Sinningia* (e.g., *S. warmingii*; Boggan 1991), and nectar has not been found in spurs of *A. grandiflora* and *A. patens* when studied in longitudinal section (E. H. Roalson, unpubl. data). Given this arrangement of tissues, it is not clear what role, if any, the spurs in *Achimenes* play in pollinator specificity.

The number of times spurs have been developed in *Achimenes* depends on the definition of a spur, that is, whether it includes corollas with a gibbose base or not. If any gibbosity at the base of the corolla is coded as a spur, then there are three derivations of this trait: once in clade 1 (*A. admirabilis*); once in clade 3 (*A. glabrata*); and once in clade 2, after *A. fimbriata* diverged from the rest of the clade, with three subsequent losses in *A. flava*, *A. occidentalis*, and *A. woodii* (Fig. 5). If, on the other hand, the definition of the spur is restricted

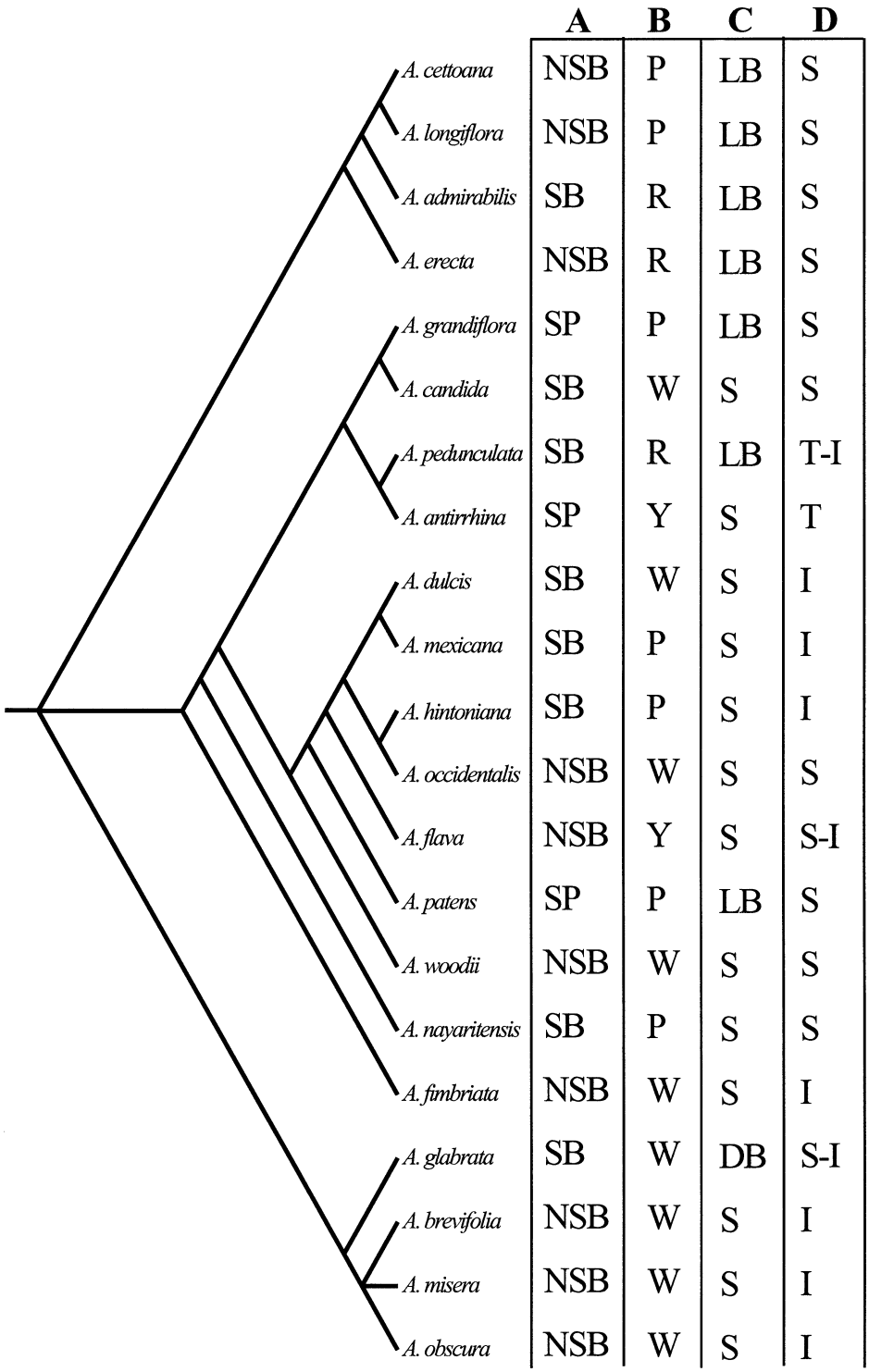

FIG. 5. Morphological characters mapped onto the phylogenetic hypothesis of relationships in *Achimenes*. A. Evolution of corolla gibbosity/spurs. Abbreviations are as follows: NSB- non-saccate base; SB- saccate base; and SP- spur. B. Variation in the primary flower color. Abbreviations are as follows: R- red; P- purple; Y- yellow; and W- white. C. Evolution of stigma shape. Abbreviations are as follows: LB- laterally bifurcate; DB- dorsiventrally bifurcate; and S- stomatomorphic. D. Evolution of flower shape. Abbreviations are as follows: S- salverform; T- tubular; and I- infundibuliform. Genus abbreviation is as follows: *A.* = *Achimenes*.

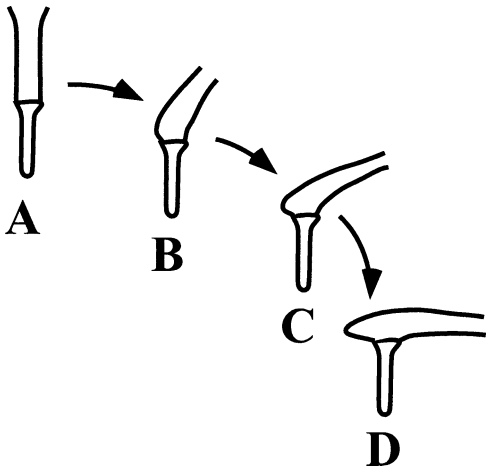

FIG. 6. Hypothetical mechanism of saccate corolla base and/or spur formation in *Achimenes*. As the corolla moves from an in-line position in relation to the flower pedicel (A) towards an oblique position in relation to the flower pedicel (D), there is an increase in the inflation of the corolla base.

to those species where there is more substantial elongation of the corolla base (say >3 mm), then spurs have apparently arisen on three independent occasions in *A. antirrhina*, *A. grandiflora*, and *A. patens*. Interestingly, the degree of corolla gibbosity is roughly correlated with the angle of presentation of the floral tube in relation to the flower pedicel (Fig. 6). That is, species in which the corolla is completely oblique in the calyx tend to have a spur, while those species where the corolla is erect in the calyx are non-saccate. This suggests that there may be a structural explanation to the variation in corolla gibbosity/spur presence. Whether spurs are the result of pollinator selection and/or structural support of the angle of flower presentation needs to be explored with field observations and consideration of the genetic control of spur development.

Evolution of Vegetative Morphology and Cytology. The vegetative morphology of *Achimenes* is quite uniform, with few characteristics obviously useful for classification above the species level. One of the few variable characters that seems congruent with the phylogenetic hypothesis is the arrangement of the leaves. Four species of *Achimenes* regularly have whorled, or more than two, leaves at a node instead of the more common opposite leaf arrangement found in the rest of the genus. These four species (*A. admirabilis*, *A. cettoana*, *A. erecta*, and *A. longiflora*) form clade 1 (Fig. 4). Leaf arrangement and the phylogeny appear to nicely split *Achimenes* into two groups: species with whorled leaves (clade 1) versus species with opposite leaves (clades 2 and 3).

There are three species with a tendency to have an anisophyllous leaf arrangement: *A. fimbriata*, *A. flava*, and *A. woodii*. This can range from one leaf being about twice the size of the other in a pair to one of the leaf pair being virtually absent (Ramírez Roa 1987). These species do not form a group and it appears that this characteristic has likely arisen on three separate occasions (Fig. 4).

Two species of *Achimenes* have a fimbriate petal margin: *A. fimbriata* and *A. glabrata*. The phylogenetic hypothesis suggests that these two species are not sister-taxa and this character has likely arisen twice.

The chromosome number of *Achimenes* is quite stable, with a few exceptions. Chromosomes have been counted for 18 of the 23 currently recognized species (Skog 1984). Of these 18 species, 14 are diploids with a haploid complement of 11 (*A. antirrhina*, *A. candida*, *A. cettoana*, *A. dulcis*, *A. fimbriata*, *A. flava*, *A. glabrata*, *A. grandiflora*, *A. heterophylla*, *A. longiflora*, *A. mexicana*, *A. obscura*, *A. patens*, and *A. woodii*), three species are tetraploids (n=22; *A. erecta*, *A. misera*, and *A. skinneri*), and two species have the odd chromosome complement of n = 17 (*A. pedunculata* and *A. skinneri* [*A. skinneri* is suggested to include both n = 17 and n = 22 chromosome races]). The phylogenetic hypothesis suggests that there were three separate tetraploidy events and one or two changes from n = 11 or n = 22 to n = 17.

While aneuploidy is common among Old World members of Gesneriaceae subfamily Cyrtandroideae (Skog 1984; Burtt and Wiehler 1995), aneuploid events appear to be much less common in the New World subfamily Gesnerioideae and there are no infrageneric aneuploid changes in the subfamily with the exception of *Achimenes* (Skog 1984; Burtt and Wiehler 1995). The counts of n = 17 were made by Fussell (1958) from horticultural material of unknown parentage. Given the prevelance of interspecific hybridizations in horticultural collections of *Achimenes* (Arnold 1969; Becker 1996), we feel the n = 17 individuals were likely stabilized hybrids (either intra- or interspecific) from n = 11 × n = 22 parentage (Fig. 7).

Biogeographic Distribution. The center of distribution of *Achimenes* is central and southern Mexico, with some species reaching as far south as northern South America (Ramírez Roa 1987). The genus is a mixture of widely-distributed species such as *A. grandiflora* and *A. longiflora* and narrow endemics such as *A. brevifolia* and *A. woodii*. As many of the species overlap in distribution (Ramírez Roa 1987), fine-scale analysis of the patterns of distribution of these species is difficult. When individual clades (Fig. 4) are considered, some conclusions may be drawn. Each clade or sub-clade (clade 1, clade 2a, clade 2b, and clade 3; Fig. 4) includes widely-distributed and narrowly endemic species (Ramírez Roa 1987). For instance, clade 1 includes the widely-distributed *A. erecta* and *A. longiflora* and the narrow endemic *A. cettoana*. Only two clades include species that have non-overlapping geographic

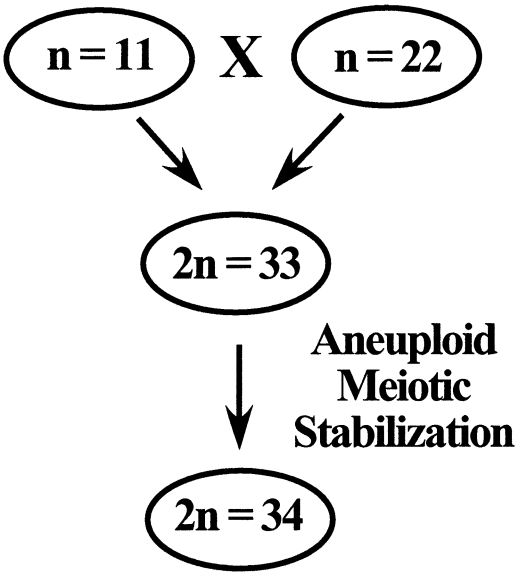

FIG. 7. Hypothesized mechanism of the derivation of the n = 17 chromosome complement in *Achimenes pedunculata* and *A. skinneri*.

distributions with the other members of their clade. These are *A. mexicana* in clade 2b and *A. misera* in clade 3. In clade 2b, five of the species overlap in distribution with at least one other species in the clade from the Mexican state of Nayarit to Chiapas (Ramírez Roa 1987), while *A. mexicana* is found in the states of Chihuahua and Sinaloa to the north (Ramírez Roa 1987). Most of the members of clade 3 are distributed from Sinaloa to Oaxaca and overlap with two of the other species, while *A. misera* is restricted to the state of Chiapas in Mexico and the Central American countries of Guatemala and Honduras (Ramírez Roa 1987). Not only do the general distributions of many of the closely related species overlap, but in many cases they also grow in the same habitat and elevational ranges, and are often found growing sympatrically (Ramírez Roa 1987). Given this information, it is not clear what, if any, biogeographic mechanisms were involved in speciation events. It may be that the species underwent allopatric speciation, with subsequent overlap in distribution. On the other hand, the shared distribution, habitat preference, and elevational range may argue more strongly for sympatric speciation driven by such mechanisms as pollinator selection.

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LITERATURE CITED

ARMBRUSTER, W. S. 1992. Phylogeny and the evolution of plant-animal interactions. BioScience 42: 12–20.

———. 1993. Evolution of plant pollination systems: hypotheses and tests with the neotropical vine *Dalechampia*. Evolution 47: 1480–1505.

ARNOLD, P. 1969. *Achimenes*, The Gesneriad Register. Gloxinian 19(Suppl.): 1–40.

ATKINS, H., J. PRESTON, and Q. C. B. CRONK. 2001. A molecular test of Huxley's line: *Cyrtandra* (Gesneriaceae) in Borneo and the Philippines. Biological Journal of the Linnean Society 72: 143–159.

BARRETT, S. C. H., L. D. HARDER, and A. C. WORLEY. 1996. The comparative biology of pollination and mating in flowering plants. Philosophical Transactions of the Royal Society of London, B 351: 1271–1280.

BECKER, J. 1996. The Gesneriad Register 1996: *Achimenes*. The American Gloxinia and Gesneriad Society, Inc. 81 + iii p.

BENTHAM, G. 1876. Gesneriaceae. Pp. 990–1025 in *Genera Plantarum 2*, G. Bentham and J. D. Hooker. London.

BOGGAN, J. K. 1991. A morphological study and cladistic analysis of *Sinningia* and associated genera with particular reference to *Lembocarpus*, *Lietzia*, *Paliavana*, and *Vanhouttea* (Gesneriaceae: Gloxinieae). M. S. Thesis, Cornell University, Ithaca, New York.

BROWNE, P. 1756. The civil and natural history of Jamaica in three parts. London: P. Browne.

BRUNNEAU, A. 1997. Evolution and homology of bird pollination in *Erythrina* (Leguminosae). American Journal of Botany 84: 54–71.

BURTT, B. L., and H. WIEHLER. 1995. Classification of the family Gesneriaceae. Gesneriana 1: 1–4.

CARPENTER, J. M. 1988. Choosing among multiple equally parsimonious cladograms. Cladistics 4: 291–296.

COOKE, J. F., and R. E. LEE. 1966. Hybridization within and between *Achimenes* P.Br. and *Smithiantha* Kuntze (Gesneriaceae). Baileya 14: 92–101.

DANDY, J. E. 1969. Nomina conservanda proposita. Taxon 18: 464–472.

DENDUANGBORIPANT, J., and Q. C. B. CRONK. 2000. High intraindividual variation in internal transcribed spacer sequences in *Aeschynanthus* (Gesneriaceae): implications for phylogenetics. Proceedings of the Royal Society of London, Series B 267: 1407–1415.

DODSON, C. H. 1962. The importance of pollination in the evolution of the orchids of tropical America. American Orchid Society Bulletin 31: 525–534.

DONOGHUE, M. J., R. H. REE, and D. A. BAUM. 1998. Phylogeny and the evolution of flower symmetry in the Asteridae. Trends in Plant Sciences 3: 311–317.

FARRIS, J. S., M. KÄLLERSJO, A. G. KLUGE, and C. BULT. 1995. Constructing a significance test for incongruence. Systematic Biology 44: 570–572.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

FEUILLET, C., and J. A. STEYERMARK. 1999. Gesneriaceae. Pp. 542–573 in *Flora of the Venezuelan Guayana, Volume 5, Eriocaulaceae-Lentibulariaceae*, eds. J. A. Steyermark, P. E. Berry, K. Yatskievych, and B. K. Holst. St. Louis, Missouri: Missouri Botanical Garden Press.

FRITSCH, K. 1893–1894. Gesneriaceae. Pp. 133–185 in *Die Natürlich-*

en Pflanzenfamilien 4(3b), A. Engler and K. Prantl. Leipzig, Germany: Wilhelm Engelmann.

———. 1897. Gesneriaceae. Pp. 299–300 in *Die Natürlichen Pflanzenfamilien 4(3b),* A. Engler and K. Prantl. Leipzig, Germany: Wilhelm Engelmann.

———. 1913. Beitrag zur Kenntnis der Gesnerioideae. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 50: 392–439.

FUSSELL, C. P. 1958. Chromosome numbers in the Gesneriaceae. Baileya 10: 33–45.

GIBSON, D. N. 1974. Flora of Guatemala, part X, number 3. Gesneriaceae. Fieldiana, Botany 24: 240–313.

GRANT, V., and K. A. GRANT. 1965. Flower pollination in the phlox family. Columbia University Press, New York, NY.

HANSTEIN, J. 1854. Die Gesneraceen des Königlichen Herbariums und der Gärten zu Berlin nebst Beobachtungen über die Familie im Ganzen. I. Abschnitt. Linnaea 26: 145–216.

———. 1856. Die Gesneraceen des Königlichen Herbariums und der Gärten zu Berlin, nebst monographischer Uebersicht der Familie im Ganzen, II. Abschnitt. Gattungen und Arten. Erstes stück. Die Niphaeen und Achimeneen. Linnaea 27: 693–785.

———. 1865. Die Gesneraceen des Königlichen Herbariums und der Gärten zu Berlin, nebst monographischer Uebersicht der Familie im Ganzen. II. Abschnitt. Gattungen und Arten. Drittes Stück. Die Eugesnereen, Rhytidophylleen und Beslerieen. Linnaea 34: 225–462.

HARRISON, C. J., M. MÖLLER, and Q. C. B. CRONK. 1999. Evolution and development of floral diversity in *Streptocarpus* and *Saintpaulia*. Annals of Botany 84: 49–60.

HILLIS, D. M., and J. J. BULL. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42: 182–192.

HODGES, S. A. 1997. Floral nectar spurs and diversification. International Journal of Plant Sciences 158(6 Suppl.): S81–S88.

JOHNSON, L. A., and D. E. SOLTIS. 1995. Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *matK* sequences. Annals of the Missouri Botanical Garden 82: 149–175.

JOHNSON, S. D., and K. E. STEINER. 1997. Long-tongued fly pollination and evolution of floral spur length in the *Disa draconis* complex (Orchidaceae). Evolution 51: 45–53.

———, H. P. LINDER, and K. E. STEINER. 1998. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). American Journal of Botany 85: 402–411.

LUCKOW, M., and H. C. F. HOPKINS. 1995. A cladistic analysis of *Parkia* (Leguminosae: Mimosoideae). American Journal of Botany 82: 1300–1320.

MANNING, J. C., and H. P. LINDER. 1992. Pollinators and evolution in *Disperis*, or why are there so many species? South African Journal of Science 88: 38–49.

MCDADE, L. A. 1992. Pollinator relationships, biogeography and phylogenetics. BioScience 42: 21–26.

MCVAUGH, R. 1970. Proposal 283. [7874] *Achimenes* Persoon (1806) vs. *Achimenes* P. Browne (1756) in Report of the Committee for Spermatophyta Conservation of Generic Names, XIII. Taxon 19: 817.

MÖLLER, M., and Q. C. B. CRONK. 1997a. Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. American Journal of Botany 84: 956–965.

———, and ———. 1997b. Phylogeny and disjunct distribution: evolution of *Saintpaulia*. Proceedings of the Royal Society of London, Series B 264: 1827–1836.

———, and ———. 2001. Evolution of morphological novelty: a phylogenetic analysis of growth patterns in *Streptocarpus* (Gesneriaceae). Evolution 55: 918–929.

———, M. CLOKIE, P. CUBAS, and Q. C. B. CRONK. 1999. Integrating molecular phylogenies and developmental genetics: a Gesneriaceae case study. Pp. 375–402 in *Molecular systematics and plant evolution,* eds. P. M. Hollingsworth, R. M. Bateman, and R. J. Gornall. London: Taylor & Francis.

MOORE, H. E. 1960. *Achimenes cettoana* (Gesneriaceae). Baileya 8: 142–145, fig. 59.

———. 1962. *Achimenes warszewicziana* and *A. misera*: two species. Baileya 10: 45.

MORTON, C. V. 1962a. A new species of *Achimenes*, section *Dicyrta*. Baileya 10: 49–52.

———. 1962b. The species of *Achimenes*, section *Dicyrta*. Baileya 10: 147–154.

NILSSON, L. A. 1988. The evolution of flowers with deep corolla tubes. Nature 334: 147–149.

PERSOON, C. H. 1806 (''1807''). *Columnea*, *Achimenes*, *Cyrtandra*, *Besleria*. Pp. 164–165 in *Synopsis Plantarum, Vol. 2.* Tubingen, Germany: J.G. Cottam.

PHILLIPS, S. M. 1970. Two new species of *Achimenes* Pers., section *Dicyrta*, from Brazil. Kew Bulletin 24: 29–34.

POSADA, D., and K. A. CRANDALL. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14: 817–818.

RAMÍREZ ROA, M. A. 1987. Revision de *Achimenes* (Gesneriaceae). Thesis, Universidad Nacional Autonoma de Mexico, D. F.

———, and L. E. SKOG. 2002. Novae Gesneriaceae Neotropicarum XII: A new species of *Achimenes* (Gesneriaceae) from Mexico. Novon 12: 382–384.

REEVES, P. A., and R. G. OLMSTEAD. 1998. Evolution of novel morphological and reproductive traits in a clade containing *Antirrhinum majus* (Scrophulariaceae). American Journal of Botany 85: 1047–1056.

SAMUEL, R., W. PINSKER, and M. KIEHN. 1997. Phylogeny of some species of *Cyrtandra* (Gesneriaceae) inferred from the *atpB/rbcL* cpDNA intergene region. Botanica Acta 110: 503–510.

SKOG, L. E. 1984. A review of chromosome numbers in the Gesneriaceae. Selbyana 7: 252–273.

———. 1987. New species of Gesneriaceae from tropical America. Opera Botanica 92: 225–233.

———, and L. P. KVIST. 2000. Revision of *Gasteranthus* (Gesneriaceae). Systematic Botany Monographs 59: 1–118.

SMITH, J. F. 1996. Tribal relationships within Gesneriaceae: a cladistic analysis of morphological data. Systematic Botany 21: 497–513.

———. 2000a. A phylogenetic analysis of tribes Beslerieae and Napeantheae (Gesneriaceae) and evolution of fruit types: parsimony and maximum likelihood analyses of *ndhF* sequences. Systematic Botany 25: 72–81.

———. 2000b. Phylogenetic resolution within the tribe Episcieae (Gesneriaceae): congruence of ITS and *ndhF* sequences from parsimony and maximum-likelihood analyses. American Journal of Botany 87: 883–897.

———. 2000c. Phylogenetic signal common to three data sets: combining data which initially appears heterogeneous. Plant Systematics and Evolution 221: 179–198.

———. 2001. The phylogenetic relationships of *Lembocarpus* and *Goyazia* (Gesneriaceae) based on *ndhF* sequences. Annals of the Missouri Botanical Garden 88: 135–143.

———, and S. ATKINSON. 1998. Phylogenetic analysis of the tribes Gloxinieae and Gesnerieae (Gesneriaceae): data from *ndhF* sequences. Selbyana 19: 122–131.

———, and C. L. CARROLL. 1997. A cladistic analysis of the tribe Episcieae (Gesneriaceae) based on *ndhF* sequences: origin of morphological characters. Systematic Botany 22: 713–724.

———, and K. J. SYTSMA. 1994. Evolution in the Andean epiphytic genus *Columnea* (Gesneriaceae). II. Chloroplast DNA restriction site variation. Systematic Botany 19: 317–336.

———, J. C. WOLFRAM, K. D. BROWN, C. L. CARROLL, and D. S. DENTON. 1997. Tribal relationships in the Gesneriaceae: evi-

dence from DNA sequences of the chloroplast gene *ndhF*. Annals of the Missouri Botanical Garden 84: 50–66.

STAFLEU, F. A., C. E. B. BONNER, R. MCVAUGH, R. D. MEIKLE, R. C. ROLLINS, R. ROSS, J. M. SCHOPF, G. M. SCHULTZE, R. DE VILMORIN, and E. G. VOSS. 1972. International code of botanical nomenclature. Regnum Vegetabile 82: 1–426.

SWOFFORD, D. 2001. PAUP*: Phylogenetic analysis using parsimony, version 4.0b6. Laboratory of Molecular Systematics, Smithsonian Institution, Washington, D.C., and Sinauer, Sunderland, MA.

TABERLET, P., L. GIELLY, G. PAUTOU, and J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17: 1105–1109.

TAMURA, K., and M. NEI. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512–526.

WIEHLER, H. 1976. A report on the classification of *Achimenes*, *Eucodonia*, *Gloxinia*, *Goyazia*, and *Anetanthus* (Gesneriaceae). Selbyana 1: 374–404.

———. 1983. A synopsis of the neotropical Gesneriaceae. Selbyana 6: 1–219.

———. 1992. New species of Gesneriaceae from the neotropics. Phytologia 73: 220–241.

———. 1995. New species of Gesneriaceae from the neotropics (II). Gesneriana 1: 29–97.

WILEY, E. O., D. SIEGEL-CAUSEY, D. R. BROOKS, and V. A. FUNK. 1991. The compleat cladist: a primer of phylogenetic procedures. The University of Kansas Museum of Natural History Special Publication No. 19. Lawrence, Kansas.

ZIMMER, E. A., E. H. ROALSON, A. IDNURM, L. E. SKOG, and J. K. BOGGAN. 2002. Phylogenetic relationships in the Gesnerioideae (Gesneriaceae) based on nrDNA ITS and cpDNA *trnL-F* and *trnE-T* spacer region sequences. American Journal of Botany 89: 296–311.