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Phylogenetic Relationships Within Nyctaginaceae Tribe Nyctagineae: Evidence from Nuclear and Chloroplast Genomes

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ABSTRACT. Nyctaginaceae are a small family of mainly New World tropical and subtropical trees, shrubs, and herbs. To date phylogenetic relationships within the family have not been examined. This study provides the first phylogenetic hypothesis of relationships within Nyctaginaceae tribe Nyctagineae based on sequence data from both nuclear (ITS) and chloroplast (*accD* 5′coding region and intergenic region between the *rbcL* and *accD* genes). Morphological characters are also discussed as they relate to the phylogeny inferred using molecular data. Results suggest that neither *Acleisanthes* nor *Selinocarpus* is monophyletic but that together they comprise a monophyletic lineage. The genus *Mirabilis* is strongly supported as monophyletic, but the monophyly of two of its sections is suspect. Morphology generally agrees with the molecular data and in some instances reinforces clades weakly supported by nuclear and chloroplast data. Further sampling will help clarify relationships of these genera within Nyctaginaceae.

Nyctaginaceae are a small family of ca. 30 genera and 400 species distributed primarily in the tropical and subtropical regions of the New World (Mabberley 1997). Monophyly of the family is suggested by two synapomorphies: the absence of a corolla (flowers possess an often petaloid, showy calyx) and a specialized anthocarp fruit-type, an achene or utricle enclosed in perianth tissue (Bogle 1974, but see discussion therein of conflicting anthocarp definitions).

Relationships within the family are less clear despite considerable morphological diversity. For instance, it appears that similar morphologies have evolved multiple times, making within-family taxonomy challenging. Perhaps as a result of these difficulties, the latest family-level revision was almost seventy years ago (Heimerl 1934). More recently an updated treatment (Bittrich and Kühn 1993) included the current literature on various genera within Nyctaginaceae, but was still based on Heimerl's (1934) classification. According to this treatment, the majority of species occur in tribes Nyctagineae (ca. 140 spp.) and Pisonieae (ca. 200 spp.).

In Nyctagineae the most species-rich subtribes are Boerhaviinae and Nyctagininae (Table 1). These two subtribes represent a segregation of Heimerl's (1934) Boerhaviinae apparently based on the presence in Nyctagininae of conspicuous, sometimes calyx-like involucral bracts subtending the inflorescence. However, the validity of such a distinction is questionable as floral bracts are present in both subtribes. Within these subtribes there have been taxonomic studies of many genera (Smith 1976; Fowler and Turner 1977; Fosberg 1978; Pilz 1978; Spellenberg 1993; Turner 1994; Le Duc 1995; Mahrt and Spellenberg 1995), although no researchers have yet taken a phylogenetic approach to the study of Nyctaginaceae at any level.

It is this paucity of knowledge of phylogenetic relationships within Nyctaginaceae that I have begun to address. I am studying phylogenetic relationships both within and among genera in Nyctagineae, with a focus on the subtribes Nyctagininae and Boerhaviinae sensu Bittrich and Kühn (1993). As part of a larger study on the relationship between the evolution of floral fragrance and hawkmoth-pollination (R. Levin, L. McDade, and R. Raguso, in mss.), I have reconstructed phylogenetic relationships within and among three genera: *Mirabilis, Selinocarpus,* and *Acleisanthes.* Based on morphology, it has been suggested that *Selinocarpus* is the closest relative of *Acleisanthes* (Smith 1976). Within *Mirabilis* there are six sections, with most species in sections *Mirabilis, Oxybaphus,* and *Quamoclidion* (Heimerl 1934; Le Duc 1995). In section *Quamoclidion* (6 spp.) there has been question about the placement of *Mirabilis triflora,* a vine with red, tubular hummingbird-pollinated flowers that is unique in the section (Pilz 1978). Pilz (1978) placed *M. triflora* within this section because it has united involucral bracts subtending the inflorescence that appear to be homologous to those that characterize the rest of section *Quamoclidion.*

These genera are mostly found in southwestern North America, with *Acleisanthes* and *Selinocarpus* mainly restricted to the Chihuahuan Desert, al-

TABLE 1. Tribe Nyctagineae sensu Bittrich and Kühn (1993). Asterisks indicate those genera included in this study. [1]Note that *Boerhavia* is diverse and often divided into multiple genera (e.g., Bittrich and Kühn 1993; Spellenberg 1993; Mahrt and Spellenberg 1995).

Genus	# spp.	Geographic Range	Source
Subtribe Nyctagininae			
**Mirabilis* L.	ca. 60	North and South America, 1 Himalayan sp.	Le Duc 1995
**Allionia* L.	1-2	SW USA to Chile	Turner 1994
Cuscatlania Standl.	1	El Salvador	Bittrich and Kühn 1993
Nyctaginia Choisy	1	Texas and Mexico	Bittrich and Kühn 1993
Subtribe Boerhaviinae			
**Boerhavia* L.[1]	ca. 50	pantropical	Mabberley 1997
**Selinocarpus* A. Gray	9	SW USA to northern Mexico, 1 Somalian sp.	Fowler and Turner 1977
**Acleisanthes* A. Gray	7	SW USA to Mexico	Smith 1976
Okenia Schdl. & Cham.	1-2	Florida to Mexico and Nicaragua	Bogle 1974; Bittrich and Kühn 1993
Caribea Alain	1	Cuba	Bittrich and Kühn 1993
Subtribe Colignoniinae			
Colignonia Endl.	6	Colombia to Argentina	Bohlin 1988
Pisoniella (Heimerl) Stand1.	1	Mexico to Argentina	Bittrich and Kühn 1993
Subtribe Phaeoptilinae			
Phaeoptilum Radlk.	1	South Africa	Bittrich and Kühn 1993

though one species of *Selinocarpus* occurs in Somalia (Thulin 1993)! Most species have long, salverform or funnelform nocturnal flowers that are primarily visited by hawkmoths (Cruden 1970; Fowler and Turner 1977; Spellenberg and Delson 1977; Pilz 1978; Grant and Grant 1983; Martinez del Río and Búrquez 1986; R. Levin, unpubl. data), although many are also capable of self-pollination (Cruden 1973; Spellenberg and Delson 1977; Martinez del Río and Búrquez 1986; Hernandez 1990). Plants of *Selinocarpus* spp. and *Acleisanthes* spp. are morphologically very similar, although *Selinocarpus* spp. are usually woody perennials, whereas *Acleisanthes* spp. are herbaceous perennials. Further, *Selinocarpus* spp. have characteristic five-winged fruits, unlike the ribbed but wingless anthocarps of *Acleisanthes* spp.

Here I present a phylogenetic hypothesis for relationships within and among *Mirabilis, Selinocarpus,* and *Acleisanthes* using DNA sequence data from both the chloroplast and nuclear genomes. This study endeavors to 1) enhance our understanding of relationships among species of *Selinocarpus* and *Acleisanthes,* 2) elucidate relationships within *Mirabilis* section *Quamoclidion* and clarify some sectional relationships within *Mirabilis,* 3) test the monophyly of these three genera, and 4) provide a preliminary test of the validity of the division of subtribe Boerhaviinae (Heimerl 1934) into subtribes Boerhaviinae and Nyctagininae (Bittrich and Kühn 1993). I will also discuss morphological characters as they relate to the phylogeny inferred using molecular data.

MATERIALS AND METHODS

Taxon Sampling. Sampling was mainly focused within *Mirabilis, Selinocarpus,* and *Acleisanthes* (Table 2). Within *Mirabilis* all six species traditionally placed into section *Quamoclidion* were included. Seven taxa representing other sections within *Mirabilis,* including sections *Mirabilis* and *Oxybaphus* (sensu Le Duc 1995) were also used to test monophyly of *Quamoclidion* and to permit polarization of character states within that section. Also included in this study were all seven species of *Acleisanthes* and eight of the nine species of *Selinocarpus.* I followed Bittrich and Kühn (1993) in considering *Ammocodon chenopodioides* (Gray) Standley as a *Selinocarpus. Selinocarpus palmeri* Hemsl. was not included because plant material was unavailable; it is only known from one locality, was last collected in 1978, and could not be found in 1998. *Allionia incarnata* was included as an outgroup within subtribe Nyctagininae, and *Boerhavia coccinea* was included as an outgroup within Boerhaviinae. The more distant outgroups *Abronia fragrans* (monogeneric tribe

TABLE 2. Taxa, voucher specimens, and GenBank accession numbers for both nuclear ITS and chloroplast (cp) sequences. Samples from species in section *Mirabilis* were made from fresh material grown from seeds of the voucher collections. BBNP is Big Bend National Park, Brewster Co., Texas. All vouchers not already labeled with location are deposited in the herbarium at the University of Arizona.

Taxon	Voucher	Locality	ITS	cp
Genus *Selinocarpus* A. Gray				
S. angustifolius Torrey	RL 97-1	BBNP, TX	AF211997	NA
S. chenopodioides A. Gray	ITS: Worthington 6694, AZ	Hudspeth Co., TX	AF211998	NA
	cp: RL 99-2	Doña Ana Co., NM	NA	AF214694
S. diffusus A. Gray	RL 98-1	Clark Co., NV	AF211991	AF214688
S. lanceolatus Wooton	RL 97-5	Culberson Co., TX	AF211995	AF214691
S. parvifolius (Torrey) Standl.	RL 97-2	BBNP, TX	AF211993	AF214689
S. purpusianus Heimerl	RL 98-6	Coahuila, MX	AF211994	AF214690
S. somalensis Chiov.	Thulin & Dahir 6517, UPS	Somalia	AF211992	AF214692
S. undulatus Fowler & Turner	RL 98-7	Coahuila, MX	AF211996	AF214693
Genus *Acleisanthes* A. Gray				
A. acutifolia Standl.	RL 98-9	Coahuila, MX	AF211988	AF214685
A. anisophylla A. Gray	RL 98-13	Coahuila, MX	AF211986	AF214683
A. crassifolia A. Gray	RL 98-12	Coahuila, MX	AF211987	AF214684
A. longiflora A. Gray	RL 97-3	BBNP, TX	AF211985	AF214682
A. nana I. M. Johnst.	RL 98-15	San Luis Potosí, MX	AF211990	AF214687
A. obtusa (Choisy) Standl.	RL 97-4	La Salle Co., TX	AF211984	AF214681
A. wrightii (A. Gray) Benth. & Hook.	RL 98-11	Coahuila, MX	AF211989	AF214686
Genus *Mirabilis* L.				
Sect. *Ouamoclidion*				
M. alipes (S. Watson) Pilz	RL 98-3	Churchill Co., NV	AF212000	AF214696
M. greenei S. Watson	RL 98-4	Siskiyou Co., CA	AF212001	AF214697
M. macfarlanei Constance & Rollins	RAR 98-101C	Ada Co., ID	AF211999	AF214695
M. multiflora (Torrey) A. Gray	RL 98-5	Pima Co., AZ	AF212002	AF214698
M. pudica Barneby	RL 98-2	Lincoln Co., NV	AF212003	AF214699
M. triflora Benth.	RL 98-23	Baja Calif. Sur, MX	AF212005	AF214701
Sect. *Oxybaphus*				
M. bigelovii A. Gray	RL 98-24	Mineral Co., NV	AF212011	NA
M. tenuiloba S. Watson	RL 98-25	Baja Calif., MX	AF212004	AF214700
Sect. *Mirabilis*				
M. jalapa L.	Le Duc 228, TEX	Puebla, MX	AF212009	NA
M. longiflora L.	Le Duc 234, TEX	Morelos, MX	AF212006	AF214702
M. polonii Le Duc	Le Duc 259, TEX	Nuevo León, MX	AF212007	NA
M. sanguinea var. *breviflora* Le Duc	Le Duc 254, TEX	Jalisco, MX	AF212008	NA
Sect. *Mirabilopsis*				
M. coccinea (Torrey) Benth. & Hook.	RL 98-20	Pima Co., AZ	AF212010	NA
Outgroups				
Allionia incarnata L.	RL 98-21	Pima Co., AZ	AF212012	NA
Boerhavia coccinea Miller	None	Pima Co., AZ	AF212013	NA
Abronia fragrans Nutt.	RAR 98-101B	Fremont Co., ID	AF212014	AF214703
Pisonia capitata (S. Watson) Stand1.	RL 99-1	UA campus	AF212015	NA

TABLE 3. Primers used for the amplification and sequencing of ca. 90 bp of *rbcL*, the intergenic region between *rbcL* and the *accD* gene, and the *accD* 5′ coding region. In the aligned sequences used in this study, Nyct-558 and Nyct-1061 are positions from the beginning of the intergenic region.

Name	Strand	Sequence	Source
1378	sense	5′-GAAGTATGGAAGGAAATCA-3′	Yasui & Ohnishi 1998
Nyct-558	antisense	5′-ACATATAGATCCAATTACTC-3′	This paper
Nyct-1061	antisense	5′-CTCTTAGATCGAGTAGTC-3′	This paper
2442	antisense	5′-GGGAATGAAGATAACTGTC-3′	Yasui & Ohnishi 1998

Abronieae sensu Bittrich and Kühn 1993) and *Pisonia capitata* (tribe Pisonieae) were also included.

For the chloroplast locus sampling differed slightly from that of the nuclear locus (Table 2). The chloroplast region was challenging to amplify because the primers on the 3′ end did not always anneal or yield a single product. Because of this amplification problem, as well as the strong support for *Mirabilis* section *Mirabilis* from the nuclear locus (see below), the taxa sampled were a subset of those studied for the nuclear locus. All taxa within *Mirabilis* section *Quamoclidion* were included as well as the seven species of *Acleisanthes* and all *Selinocarpus* except *S. angustifolius* and *S. palmeri.* Two taxa representing two different sections of *Mirabilis* were also included. *Abronia fragrans* was used as the outgroup; the other outgroups used for the nuclear locus were not included because they did not amplify for the chloroplast locus.

DNA Extraction and Amplification. Fresh or silica gel-dried material was available for all but two taxa; for these herbarium material was used (Table 2). Total genomic DNA was extracted using the modified CTAB method of Doyle and Doyle (1987).

NUCLEAR LOCUS. PCR amplification of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA, composed of ITS-1, the 5.8S gene, and ITS-2 (Baldwin 1992; Baldwin et al. 1995), used the primers C26A and N-nc18s10 (Wen and Zimmer 1996). PCR conditions varied but mainly consisted of a ''touchdown'' procedure, with an initial annealing temperature of 58°C, then a decrease to 56°C, then to 54°C with two cycles at each temperature. Subsequently the temperature was decreased by 1°C every two cycles until 49°C. At 49°C thirty amplification cycles were conducted. This procedure yielded a single product of ca. 700 nucleotides in length.

CHLOROPLAST LOCUS. PCR amplification of an approximately 1100 base pair (bp) sequence used the primers listed in Table 3. This sequence includes ca. 90 bp at the 3′ end of *rbcL,* the intergenic region between the 3′ *rbcL* gene and the *accD* gene, and the *accD* 5′ coding region (encodes one of the subunits of acetyl-CoA carboxylase; Yasui and Ohnishi 1998). Most taxa were amplified using primer 1378 at the 3′ end of *rbcL* (Yasui and Ohnishi 1998) and primer Nyct-1061 at the 5′ end of *accD* (instead of Nyct-1061 a few were amplified with primer 2442 of Yasui and Ohnishi 1998) using an annealing temperature of 52°C and 40 amplification cycles. I was unable to use these primer pairs for amplification in some of the taxa. Consequently, for these taxa a shorter region was amplified using primer 1378 (Yasui and Ohnishi 1998) and Nyct-558, a primer positioned at the 3′ end of the intergenic region.

Sequencing. Cleaned PCR products (QIAquick™ PCR purification kit, QIAGEN) were sequenced in both directions. Sequencing used the same primers as for amplification and the Big-Dye Ready Reaction Kit (Perkin-Elmer). Sequencing reactions consisted of 25 cycles and were conducted on an ABI automated sequencer at the University of Arizona DNA sequencing facility.

Alignment and Analysis. Sequences were edited and aligned using AutoAssembler™ DNA Sequence Assembly Software version 1.4.0 (Applied Biosystems 1989–95) and SeqApp (Gilbert 1993). Character by taxon matrices were prepared in MacClade (Maddison and Maddison 1999).

PAUP* (Swofford 1998) was used to reconstruct phylogenetic relationships among all 32 taxa for which ITS sequence data were available. Sequences in the data matrix had an average of 0.0094% missing data, and an average of 4.53% gaps (indels). Parsimony analyses were conducted using the heuristic search option and 100 random addition sequence replicates with tree-bisection reconnection (TBR) branch-swapping. Gaps were treated as missing data. Analyses were also done including extra characters for the presence/absence of >1 bp indels. Multiple equally parsimonious trees were combined as a strict consensus tree.

Phylogenetic reconstruction of the 23 taxa for

which chloroplast data were collected was similar to above. For four taxa I obtained partial sequences such that there was an average of 39.4% missing data and 4.63% gaps. For the remaining 19 taxa there was an average of 0.021% missing data and 6.57% gaps. As the number of parsimony informative characters was very low in comparison to the entire data set, I used only the parsimony informative characters and conducted branch and bound searches. Gaps were treated as missing data. Analyses were also done including extra characters for the presence/absence of >1 bp indels. Multiple most parsimonious trees were combined in a strict consensus tree.

A partition homogeneity test (Farris et al. 1995) was conducted (PAUP*; Swofford 1998) to determine the congruence of the chloroplast and nuclear data sets. For this test 100 replicates were performed, using the heuristic search option with simple addition, TBR branch-swapping, and gaps treated as missing data. Because of memory constraints only variable characters were included.

Data sets were combined for the 23 taxa for which both nuclear and chloroplast data were available. Parsimony analyses were conducted using the branch and bound search option, with gaps treated as missing data. Analyses were also done including extra characters for the presence/absence of >1 bp indels. Multiple equally parsimonious trees were combined as a strict consensus tree.

For the ITS and combined data sets, the strength of support for individual tree branches was estimated using bootstrap values (BS) (Felsenstein 1985) and decay indices (DI) (Bremer 1988; Donoghue et al. 1992). Bootstrap values were from 100 full heuristic replicates (150 for ITS) with 100 random addition sequences (10 for ITS), TBR branch swapping, and the MulTrees option that saves all minimal trees. Decay values for each branch were determined by first using the decay index PAUP file command in MacClade (Maddison and Maddison 1999) to prepare a set of trees each with a single branch resolved. This file was then executed in PAUP* (Swofford 1998) using the heuristic search option to find the shortest trees consistent with each constraint. The decay index for each branch in question is the difference in length between the shortest trees consistent with a particular constraint and the globally shortest trees.

Constraint trees were constructed in MacClade (Maddison and Maddison 1999) to test various hypotheses of phylogenetic relationships. These trees were then loaded into PAUP* (Swofford 1998) and branch and bound searches were conducted to find the shortest trees consistent with each constraint. The additional steps required for a particular constraint are given by the difference in length between the shortest trees consistent with that constraint and the globally shortest trees.

Maximum likelihood (ML) analyses were also conducted for each data set and the combined data set using PAUP* (Swofford 1998). Nucleotide frequencies were empirically determined. ML analyses were begun to estimate the transition:transversion ratio and rates of evolution. These analyses were stopped before completion and the estimated transition:transversion ratio and gamma distribution shape parameter were then specified in analyses that were completed.

RESULTS

Nuclear. Aligned length for the entire ITS region was 690 bp. There were four >1 bp indel characters that were also added to the data set. The inclusion of these indels had no effect on the overall tree topology, and this analysis will not be discussed further. The ITS data included 158 parsimony informative characters and yielded 260 most-parsimonious trees. The strict consensus of these trees (Fig. 1) shows a highly supported clade composed of *Acleisanthes* and *Selinocarpus* (*Acleisanthes* lineage; BS=100, DI=15) but does not support the monophyly of each genus individually. However, there is strong support for the clade composed of *A. obtusa, A. longiflora,* and *A. anisophylla* (*Acleisanthes* A; BS=94, DI=3), as well as for a clade of the remaining four species of *Acleisanthes* and all *Selinocarpus* (*Acleisanthes* B; BS=88, DI=3). Additionally, within this last clade there is strong support for a clade composed of *A. acutifolia, A. nana, A. wrightii,* and *S. diffusus* (BS=98, DI=5).

The monophyly of *Mirabilis* is well supported (BS=100, DI=16), and *Mirabilis* section *Quamoclidion* is supported as a monophyletic group excluding *Mirabilis triflora* (BS=90, DI=3). Further, *Mirabilis* section *Mirabilis* is confidently placed as a monophyletic lineage (BS=99, DI=7). *Boerhavia coccinea* and *Allionia incarnata* are supported as each other's closest relative in this analysis (BS=81, DI=3).

Chloroplast. The aligned length for 90 bp at the 3′ end of *rbcL,* the intergenic region between *rbcL* and *accD,* and ca. 300 bp at the 5′ end of the *accD* gene was 1101 bp. These data on their own yielded very little resolution (topology not shown), because

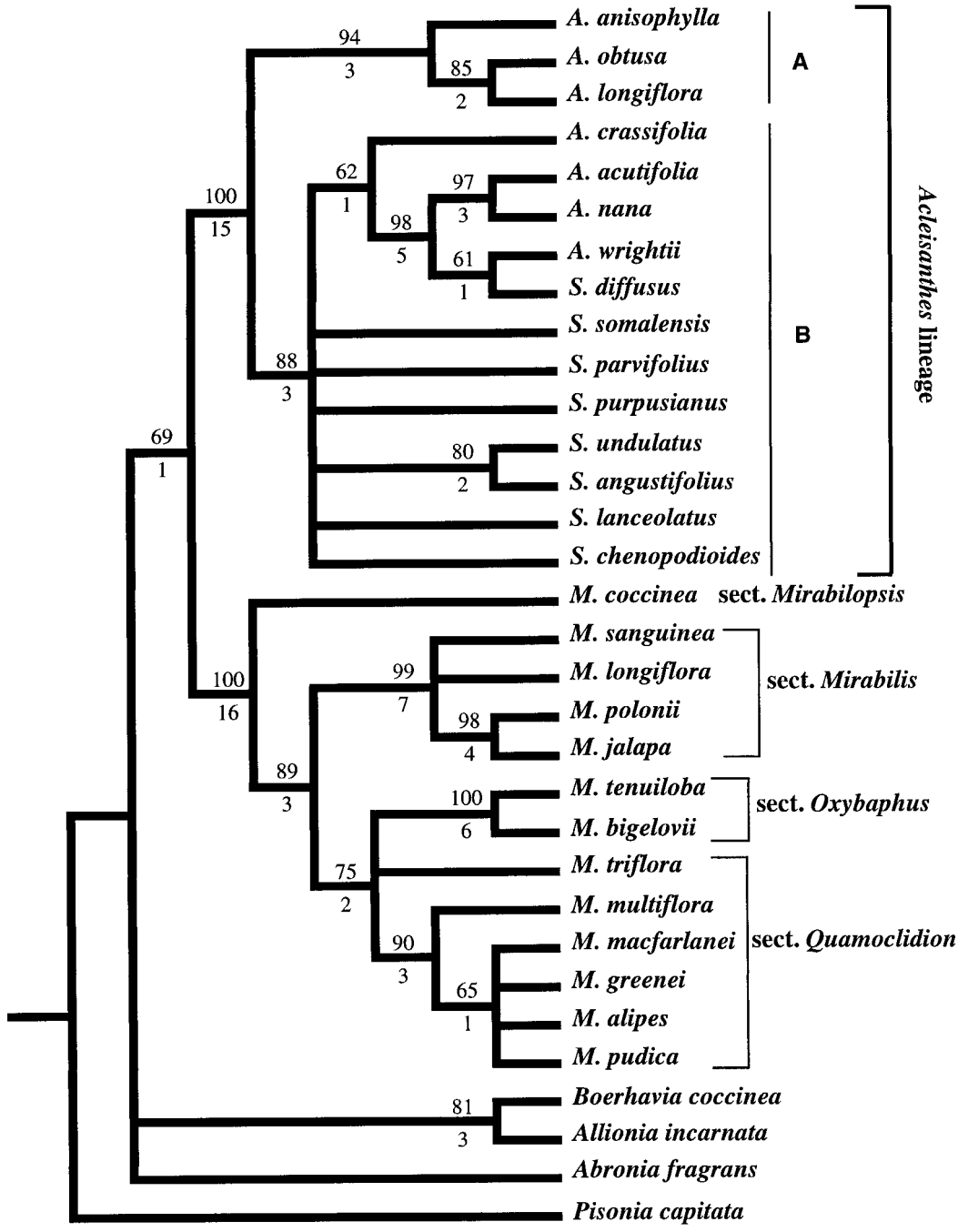

FIG. 1. The strict consensus of 260 most-parsimonious trees using the ITS data (TL=544, CI=0.73, RI=0.86). The numbers above the branches are bootstrap values and the numbers below are decay indices. A and B indicate the *Acleisanthes* A and *Acleisanthes* B clades within the *Acleisanthes* lineage.

there were only 28 parsimony informative characters, not including indels. However, they did yield better resolution within *Mirabilis* section *Quamoclidion* than ITS, suggesting that *M. macfarlanei* is sister to a clade composed of *M. greenei* and *M. multiflora.*

Combined. Almost all the random partitions of the data had summed lengths greater than the summed length of the original partition. However, the lengths of the random partitions exceeded the summed length of the original partition by only 0.2–1.2% (i.e., maximally 5 steps longer). Therefore, the nuclear and chloroplast data were combined. Including indels there were 1811 characters, 134 of which were parsimony informative. The strict consensus of 15 trees is shown in Fig. 2.

There is strong support for monophyly of *Acleisanthes* plus *Selinocarpus* (*Acleisanthes* lineage; BS=100, DI=30) and for monophyly of *Mirabilis* (BS=100, DI=32). Within the *Acleisanthes* lineage there is moderate support for *Acleisanthes* A composed of three species of *Acleisanthes* (BS=87, DI=2) and strong support for the *Acleisanthes* B clade that includes the rest of *Acleisanthes* and all *Selinocarpus* (BS=100, DI=8). Within *Acleisanthes* B there is moderate support for a clade composed of the other four species of *Acleisanthes* and *Selinocarpus diffusus* (BS=87, DI=3), with very strong support for *Acleisanthes* B above *Acleisanthes crassifolia* (BS=100, DI=5). Relationships are less clear among other members of *Acleisanthes* B, although there is moderate support for sister taxon relationships between *Selinocarpus parvifolius* and *S. lanceolatus* (BS=83, DI=2) and *S. somalensis* and *S. chenopodioides* (BS=70; DI=2).

In *Mirabilis* there is strong support for a clade composed of taxa traditionally placed in section *Quamoclidion* plus *M. tenuiloba* (BS=100, DI=6). Additionally, there is moderate support for a clade composed of section *Quamoclidion* as circumscribed by Pilz (1978) but excluding *Mirabilis triflora* (BS=71, DI=2).

ML versus Parsimony. Maximum likelihood analyses yielded tree topologies (not shown) that were a subset of the resultant topologies assuming parsimony. The main difference was that ML searches found fewer optimal tree topologies than parsimony.

Discussion

Molecular Evolution. By comparing the nuclear ribosomal ITS region with the chloroplast locus, it is clear that in Nyctaginaceae ITS evolves much faster than the intergenic region between *rbcL* and *accD* (IGR) and the 5′ *accD* coding region (Table 4). Although comparable in length, the ITS region has five times the number of parsimony informative characters as the IGR, and over eight times that of the much shorter 5′ *accD* region. Despite the large number of changes, the ITS region is not much more homoplastic than the chloroplast regions, as indicated by the high consistency indices for all three regions (Table 4). It therefore appears that the chloroplast IGR and 5′ *accD* coding region are not as phylogenetically informative for deducing patterns of relationships among species and closely related genera as is the nuclear ITS region. This result might be expected as most of the ITS region is noncoding. However, it should be noted that the amount of the 5′ *accD* region that is completely coding (i.e., does not contain internal stop codons) has been reduced in many of the taxa included in this study, suggesting that this region is no longer fully functional.

The results for Nyctaginaceae are in contrast to the phylogenetic utility of these chloroplast regions among species of *Fagopyrum* (Polygonaceae)(Yasui and Ohnishi 1998). What may explain this discordance is that the 5′ *accD* region sequenced by Yasui and Ohnishi (1998) is twice as long as that for Nyctaginaceae (due at least in part to different primers). The *accD* gene, which is 1539 bp long in *Nicotiana* (Shinozaki et al. 1986), is thought to have a very divergent 5′ region and a more conserved 3′ region (Doyle et al. 1995). This suggests that the 5′ region should be phylogenetically informative in Nyctaginaceae and other plant families; the challenge is to find conserved primer sites in such a variable area.

The number of scored indels is also notable among the nuclear ITS and chloroplast regions (Table 4). The chloroplast IGR has three times more scored indels than does the nuclear ITS region. Further, the IGR has four times more scored indels than the 5′ *accD* region, although the 5′ *accD* region has more variable sites, parsimony informative sites, and distance between taxa. These differences help explain the relative ease in locating primers in the IGR versus the 5′ *accD* region. The number of indels in both the IGR and 5′ *accD* regions is similar to that observed in *Fagopyrum* (Yasui and Ohnishi 1998).

Acleisanthes Lineage. The combined molecular evidence strongly supports the monophyly of *Acleisanthes* plus *Selinocarpus,* but not the monophy-

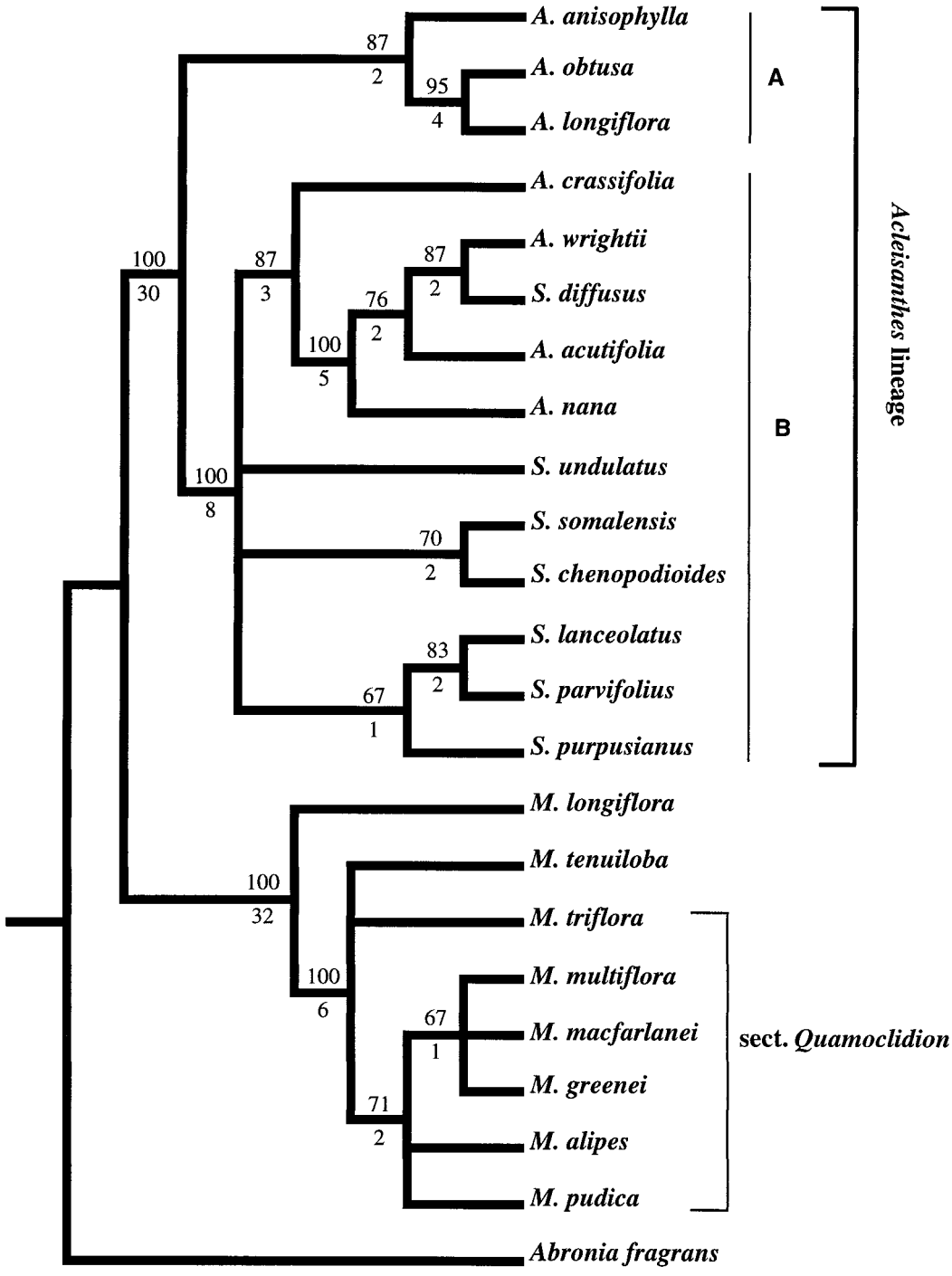

FIG. 2. The strict consensus of 15 most-parsimonious trees using the combined data set of nuclear and chloroplast data and >1 bp indels (TL=406, CI=0.87, RI=0.94). The numbers above the branches are bootstrap values and the numbers below are decay indices. A and B indicate the *Acleisanthes* A and *Acleisanthes* B clades within the *Acleisanthes* lineage.

TABLE 4. Comparison of the nuclear ribosomal ITS region and the chloroplast locus differentiated into the intergenic region between the *rbcL* and *accD* genes (IGR) and the 5′ *accD* coding region. Pairwise distances were calculated using an HKY85 distance metric (PAUP*; Swofford 1998). Only the 19 taxa for which sequences were available for all three regions were included. *nr-ITS region includes 25 bp of the 18S and 65 bp of the 26S genes in addition to ITS-1, ITS-2, and the 5.8S gene.

	IGR	5′ *accD*	nr-ITS region*
Range of raw length	614-640	297-331	655-665
Aligned length	670	333	690
Variable sites (proportion)	44 (0.066)	38 (0.114)	171 (0.248)
Parsimony informative sites (proportion)	17 (0.025)	10 (0.030)	88 (0.128)
Pairwise distances	0-3.313%	0-7.159%	0.152-17.41%
Scored indels	12	3	4
Consistency index	0.96	0.97	0.87

ly of the two genera as currently circumscribed. For these two genera to each be monophyletic, it would require 23 more steps (~6% longer). These molecular results are consistent with the similar floral morphology of these two genera. Further, members of both genera often produce both cleistogamous and chasmogamous flowers on the same individual. Within this lineage there is strong support for *S. diffusus* nested within a clade of four species of *Acleisanthes* (although not included in the analyses presented here, another individual of *S. diffusus* was also sequenced and yielded the identical result). This was a surprising finding as *S. diffusus* has winged anthocarp fruits similar to those found in all other members of *Selinocarpus.* However, winged anthocarp fruits have clearly evolved multiple times within the family; they are known in *Selinocarpus* and *Boerhavia* (Nyctagineae: Boerhaviinae), *Colignonia* (Nyctagineae: Colignoniinae), *Phaeoptilum* (Nyctagineae: Phaeoptilinae), *Abronia* Juss. (tribe Abronieae), and *Grajalesia* Miranda (tribe Pisonieae) (Willson and Spellenberg 1977; Bittrich and Kühn 1993). Further morphological evidence for the phylogenetic placement of *S. diffusus* is its herbaceous habit, similar to *Acleisanthes,* rather than the woody habit of most *Selinocarpus* (Fig. 3). In addition, it has been previously noted that vegetatively *A. wrightii* and *S. diffusus* look identical (Smith 1976). Fifteen more steps (~4% longer) are required for *Selinocarpus* to form a monophyletic group containing *S. diffusus.*

The phylogenetic relationships within *Acleisanthes* are concordant with morphology (Fig. 3). The basal *Acleisanthes* A clade including *A. obtusa, A. longiflora,* and *A. anisophylla* all lack resinous glands on their anthocarps, whereas the other *Acleisanthes* except for *A. crassifolia* have resinous glands (*S. diffusus* does not have these glands). Further, plants of *Acleisanthes* A produce cleistogamous flowers that have five stamens rather than the usual two stamens found in cleistogamous flowers in the rest of the genus as well as in many *Selinocarpus* including *S. diffusus* (Smith 1976; R. Levin, unpubl. data). Additional morphological support for these relationships comes from the strong vegetative and floral similarities between *A. wrightii* and *A. acutifolia,* the main distinction being a slight difference in the location of the resinous glands on the fruit and a more robust growth habit in *A. acutifolia. Acleisanthes crassifolia* has traditionally been considered intermediate between the resinous gland clade and the glandless clade (Smith 1976); although it lacks resinous glands, its cleistogamous flowers possess only two stamens. Thus, morphology is concordant with the position of *A. crassifolia* as basal to the resinous gland clade.

The traditionally circumscribed *Selinocarpus* excluding *S. diffusus* (=*Selinocarpus* sensu stricto) may be monophyletic and nested within a polyphyletic *Acleisanthes*; constraining *Selinocarpus* s.s. as monophyletic requires only one additional step. Alternatively, for monophyly of *Selinocarpus* s.s. and monophyly of *Acleisanthes* plus *S. diffusus* there is a cost of eight additional steps (2% longer). Within *Selinocarpus* s.s. there is limited molecular and morphological evidence for relationships among taxa. However, the ITS data (Fig. 1) do suggest a sister taxon relationship between *S. angustifolius* (cp data were not available for this species) and *S. undulatus,* which is consistent with morphology. Plants of the two species are only distinguishable by the undulating leaf margins of *S. undulatus,* and this species previously has been considered a subspecies of *S. angustifolius* (Fowler and Turner 1977). Further, al-

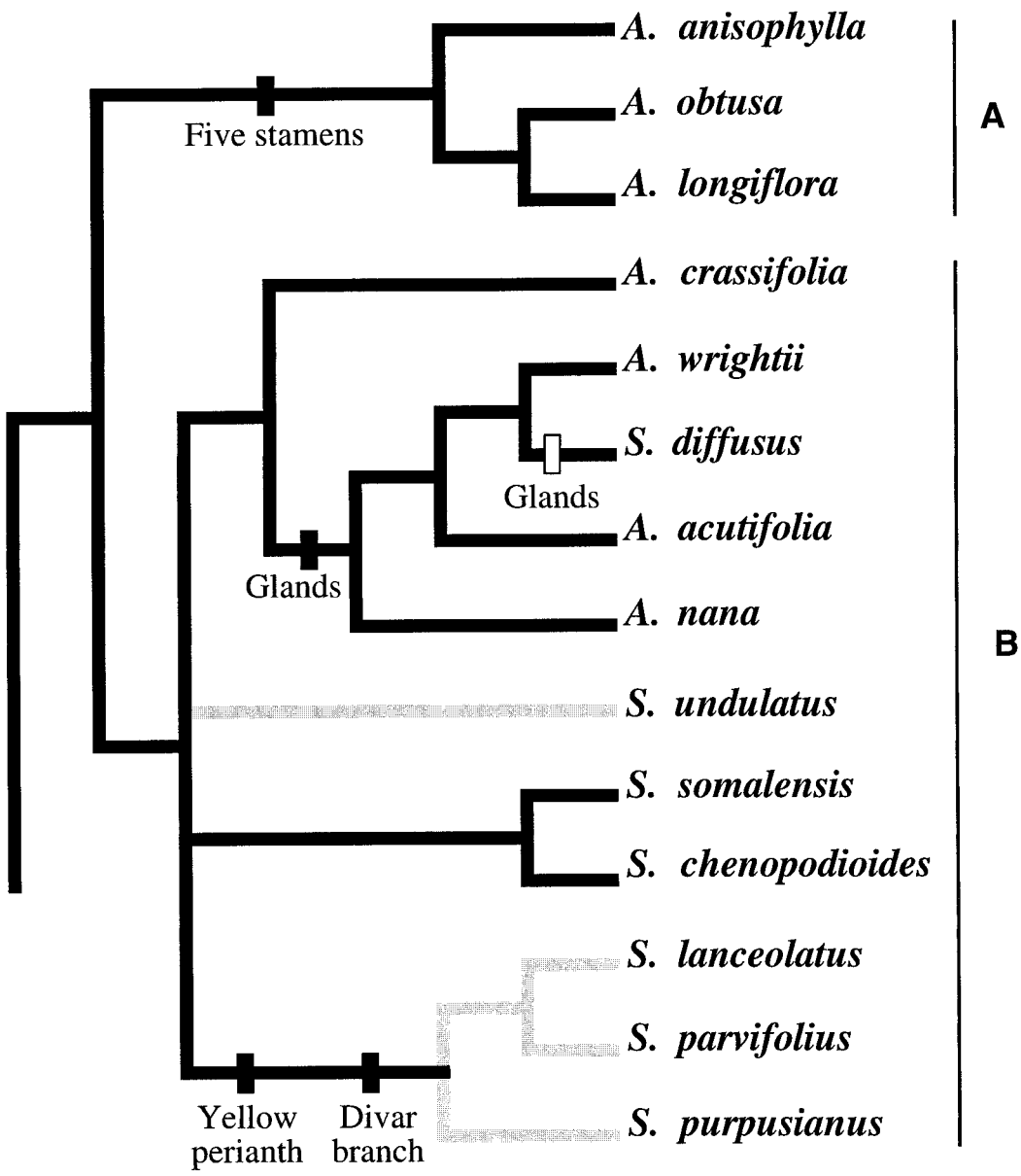

FIG. 3. *Acleisanthes* lineage from Fig. 2 with morphological characters that support clades within this lineage. The woody habit is indicated by gray shading; all other taxa are herbaceous (unclear for *S. somalensis*). Closed bars indicate character gain and open bars indicate loss. The *Acleisanthes* A clade shares five stamens in their cleistogamous flowers. Glands are resinous glands on the anthocarp; Divar branch is a divaricating branching pattern of plant form.

though support for a clade composed of *S. parvifolius, S. lanceolatus,* and *S. purpusianus* is weak (Fig. 2; BS=67, DI=1), there is strong morphological evidence for this clade, including a yellow perianth and divaricating branching pattern unique to this three-membered clade (Fig. 3). Floral fragrance data (R. Levin, R. Raguso, and L. McDade, in mss.) further corroborate this clade as well as the sister taxon relationship between *S. parvifolius* and *S. lanceolatus.*

Because of its unique floral and inflorescence morphology, *Selinocarpus chenopodioides* A. Gray has

often been considered as the sole member of its own genus *Ammocodon* (Standley 1916; Fowler and Turner 1977). However, I agree with its original placement as a member of *Selinocarpus.* Molecular data support its phylogenetic position within *Selinocarpus* s.s. *Selinocarpus somalensis* is also well supported as a member of this genus, laying to rest doubts about the proper placement of this Somalian endemic in an otherwise North American clade. Further, this taxon does not appear to be sister to the North American *Acleisanthes* lineage (requires 10 more steps, 2.5% longer), suggesting that long distance dispersal is the most reasonable explanation for its disjunct occurrence in Somalia. This is in contrast to Thulin (1994), who explains this disjunct distribution by vicariance combined with extinction events.

Mirabilis. Molecular evidence strongly supports monophyly of the genus *Mirabilis.* This is consistent with the presence of often calyx-like involucral bracts subtending one to many flowered inflorescences. Although only four of ten taxa from section *Mirabilis* were included, this is a relatively homogeneous section. Therefore, I would suggest that there is strong molecular support for the monophyly of section *Mirabilis.* This is consistent with the shared presence of one flower per involucre, creating the appearance of a calyx subtending a corolla (Le Duc 1995).

The monophyly of section *Quamoclidion* sensu stricto (i.e., excluding *M. triflora*) is also well supported by both molecular and morphological data. All *Quamoclidion* s.s. share an inflorescence composed of five partially united bracts surrounding six or more flowers; most often there is one whorl of six flowers composed of a central solitary flower surrounded by five others attached at the bases of the five involucral bracts. In *M. triflora,* inflorescences have only three flowers arranged as a solitary central flower and two borne at the bases of the two largest involucral bracts. This morphology is in fact more similar to that of some species traditionally placed in section *Oxybaphus* [e.g., *M. pumila* (Standley) Standley]. It is unclear from my results whether *M. triflora* is basal to *Quamoclidion* s.s. or if it is actually more closely related to species in section *Oxybaphus.* Section *Oxybaphus* may contain ca. 40 species and is diverse, with both single-flowered and multiple-flowered inflorescences found in the section. This diversity in inflorescence structure suggests that section *Quamoclidion* may be nested within *Oxybaphus.* However, increased sampling of species from section *Oxybaphus* is needed to establish the phylogenetic status of *Oxybaphus* and placement of *Quamoclidion* with respect to members of this large section.

Within section *Quamoclidion* s.s., there is no molecular support, but good morphological evidence for a sister relationship between *M. alipes* and *M. pudica.* Plants of these species have almost identical campanulate flowers. The shared presence of linalool oxides in their floral fragrance further supports this relationship (R. Levin, R. Raguso, and L. McDade, in mss.). Similarly, although the clade of *M. multiflora, M. macfarlanei,* and *M. greenei* is weakly supported with molecular data (BS=67, DI=1), all three share magenta, funnelform flowers and are very similar vegetatively.

Higher Relationships. The results presented here suggest that the division of subtribe Boerhaviinae (Heimerl 1934) into subtribes Boerhaviinae and Nyctagininae (Bittrich and Kühn 1993) is not warranted. *Allionia* (Nyctagininae) and *Boerhavia* (Boerhaviinae) are more closely related to each other than to either *Mirabilis* or the *Acleisanthes* lineage (Fig. 1). To support the subtribal circumscription of Bittrich and Kühn (1993), *Boerhavia* should be more closely related to the *Acleisanthes* lineage than it is to either *Allionia* or *Mirabilis.* When *Boerhavia* was constrained to be a member of the *Acleisanthes* lineage and *Allionia* more closely related to *Mirabilis,* there were an additional 161 steps required (~40% longer). However, before strong conclusions can be made at the subtribal level, more taxa must be included, with increased sampling within *Boerhavia* and the inclusion of *Nyctaginia, Okenia, Caribea,* and *Cuscatlania.* Further, species in the genera *Colignonia, Pisoniella,* and *Phaeoptilum* should also be included from the other subtribes within Nyctagineae. We now have a phylogenetic understanding of major genera within Nyctagineae. Increased sampling from all tribes and genera of Nyctaginaceae should further clarify relationships in the entire family.

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