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Phylogenetic Relationships in *Solanum* Section *Androceras* (Solanaceae)

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Abstract—The Leptostemonum clade of *Solanum* contains approximately 350–450 species, including the cultivated eggplant, *S. melongena*. This clade is characterized by the presence of prickles and apically attenuate anthers. *Solanum* section *Androceras*, the focus of this study, is a group of ca. 12 species belonging to the Leptostemonum clade. This section is unusual in the genus because of its mostly north temperate distribution and distinctive zygomorphic, heterantherous, and enantiostylous flowers. We infer phylogenetic relationships among 43 *Solanum* taxa, including 11 species and all varieties of sect. *Androceras*, using DNA sequence data from two nuclear regions (ITS and the granule-bound starch synthase gene [GBSSI or *waxy*]) and the chloroplast region *trnT-F*. The combined phylogenetic tree supports sect. *Androceras* as a monophyletic group sister to *Solanum* sect. *Crinitum*. Only one of the three series proposed by previous taxonomists, ser. *Pacificum*, is supported as monophyletic. *Solanum tenuipes* from the northern Chihuahua Desert is sister to the remaining species in sect. *Androceras*. Species-level relationships were also examined and it was found that two species, *S. heterodoxum* and *S. citrullifolium*, are not monophyletic. The ancestral flower color in sect. *Androceras* appears to be violet, with white and yellow flowers restricted to more derived clades. Characters formerly used to diagnose ser. *Androceras*, such as exclusively branched hairs and lack of complex foliar flavonoids, appear to have evolved more than once in the section.

Keywords—enantiostyly, heteranthery, ITS, Mexico, *trnT-F*, *waxy*.

Solanum L. (Solanaceae), thought to contain approximately 1,400 species, is one of the 10 largest genera of flowering plants (Frodin 2004; Bohs 2005). It also contains economically important species such as the tomato (*S. lycopersicum* L.), eggplant (*S. melongena* L.), and potato (*S. tuberosum* L.). Recent studies of the genus range from sequencing the genome of the tomato (Mueller et al. 2005; http://www.sgn.cornell.edu/) to resolving phylogenetic relationships within *Solanum* as well as species level taxonomy (Knapp et al. 2004; http://www.nhm.ac.uk/solanaceaesource/). With respect to the phylogeny of the genus, analyses of DNA sequence data have helped to identify the major groups within *Solanum*, the largest of which is the Leptostemonum clade with approximately 350–450 species (Bohs 2005; Levin et al. 2006). This group is commonly known as the "spiny solanums" due to the presence of sharp epidermal prickles.

Within the Leptostemonum clade, *Solanum* sect. *Androceras* is unique in many features including distribution, flower and fruit morphology, and chemistry. Its morphological characteristics, specifically floral morphology, are so distinct that Nuttall (1818) placed the species in the genus *Androcera* Nutt., although he noted the similarities between *Androcera* and *Solanum*. Marzell (1927) placed the species of *Androcera* into *Solanum* sect. *Androceras*. Whalen (1979a) provided a detailed revision of *Solanum* sect. *Androceras*, including 12 species and 10 varieties, and divided the section into three series (discussed below; Table 1) based on hair, flower, seed, and chemical characteristics as well as geographical distributions. Species in the section range from the midwestern U. S. A. through Mexico to Honduras, with the highlands around Mexico City, the northern Chihuahuan Desert, and the west coast of Mexico as centers of diversity (Table 1). This section is one of the only groups in *Solanum* to have a primarily north temperate distribution. Within its range, species of sect. *Androceras* are weedy annual herbs or perennials from persistent woody roots. Many species grow in warm, semiarid to arid regions with unpredictable seasonal rainfall. Chromosome counts have been reported for all species in sect. *Androceras*, and all are diploids with $2n = 24$ (Whalen 1979a).

Typical *Solanum* flowers are radially symmetrical with stamens dehiscing by terminal pores. They are usually buzz pollinated, ejecting pollen from the pores when vibrated by bees. Species in sect. *Androceras* conform to this basic plan, but are further specialized in being bilaterally symmetrical. The stamens within a single flower are unequal in size, with four small, straight upper anthers and an elongate lower anther (heteranthery; Bohs et al. 2007). This elongated, inwardly-curved lowermost stamen can be a different color than the other stamens and is opposed by a slender style of similar shape (Fig. 1a, b, c). The position of the style alternates between the right and left side of the flower along the inflorescence, resulting in "mirror-image" flowers (enantiostyly).

Flowers of sect. *Androceras*, specifically *S. rostratum*, have been extensively observed in field and natural history studies with a focus on the unusual stamen dimorphism (Todd 1882; Harris and Kuchs 1902; Bowers 1975; Jesson and Barrett 2002). The upper four small stamens provide the pollen that the bees use for food, whereas the lowermost, elongated stamen acts as a pollinating stamen by depositing pollen on one side of the bee's abdomen where it cannot efficiently be removed (Bowers 1975; Vallejo-Marin et al. 2009). The alternating right- and left-handed flowers have been shown to have higher outcrossing rates than plants manipulated to have either straight styles or right-handed or left-handed flowers only (Jesson and Barrett 2002). This might be especially important in maintaining genetic diversity in sect. *Androceras*, where all tested species have been found to be self-compatible (Whalen 1979a).

Most species of *Solanum* have fleshy berries, whereas fruits in sect. *Androceras* are dry at maturity and tightly enveloped by a prickly, accrescent calyx (Fig. 1d). Whalen (1979a) showed that these represent a "censer" dispersal mechanism, also seen in other members of the Leptostemonum clade, particularly those of dry habitats, in which the fruits remain on the plant and the calyx splits open, tearing the dry berry (Symon 1984; Knapp 2002). This then acts like a "censer," shaking loose the small seeds. The large number of seeds produced by a single plant, in some cases over 5,000 seeds from an individual, corresponds to the observation that *Solanum* sect. *Androceras* is typically a weedy, colonizing group of species.

Some species of sect. *Androceras* have a unique suite of flavonoid compounds, such as 8-hydroxyflavonoids and C-glycosylflavones, not found in other *Solanum* groups (Whalen 1978a). Differences also exist in the chemical profiles between the three series within the section recognized

TABLE 1. Species of *Solanum* sect. *Androceras*, including the series and their distributions according to Whalen (1979a). All taxa except *S. leucandrum* were sampled in this study.

Solanum section *Androceras* (Nutt.) Marzell	Geographic Distributions
Series *Androceras*	
S. angustifolium Mill.	Tropical Mexico south to Honduras
S. fructo-tecto Cav.	Distrito Federal, Hidalgo, and México States with collections from Ciudad Durango and the Sierra Madre, Mexico
S. johnstonii Whalen	Endemic to eastern Durango State, Mexico
S. rostratum Dunal	Widespread from Mexico City through the Great Plains, U.S.A.; introduced worldwide
S. tribulosum Schauer	Querétaro to southeastern Puebla State, Mexico
Series *Pacificum* Whalen	
S. grayi Rose var. *grayi*	Southern Sonora and northern Sinaloa, Mexico
var. *grandiflorum* Whalen	Southern Sinaloa and south along the Sierra Madre, east to Guerrero inland in central Mexico to Morelos
S. leucandrum Whalen	Known only from the type locality in western Puebla, Mexico
S. lumholtzianum Bartlett	Southern Arizona, and Sonora to northern Sinaloa, Mexico
Series *Violaceiflorum* Whalen	
S. citrullifolium A. Braun var. *citrullifolium*	North-central Coahuila, Mexico to the Davis Mts. of western Texas, with a cluster of populations in central Texas
var. *knoblochii* Whalen	Known only from two localities in Tarahumara country of western Chihuahua, Mexico
var. *setigerum* Bartlett	Eastern Chihuahua and western Coahuila, occasionally Presidio County, Texas
S. davisense Whalen	Davis, Chinati, and Chisos Mts. of west Texas and Sierra del Carmen in northern Coahuila, Mexico
S. heterodoxum Dunal var. *heterodoxum*	Veracruz northwest across Puebla and Hidalgo to San Luis Potosí, Mexico
var. *novomexicanum* Bartlett	Mountains of north-central New Mexico
var. *setigeroides* Whalen	Northern Chihuahua, southeastern Arizona, and southwestern New Mexico
S. tenuipes Bartlett var. *tenuipes*	Eastern Coahuila State, Mexico to Brewster, Terrel, Val Verde, and Maverick Counties, Texas
var. *latisectum* Whalen	Presidio County, Texas south along the Chihuahua and Coahuila borders to eastern Durango, Mexico

by Whalen (1979a), such as the presence of methoxylated aglycones, 8-hydroxyflavonoids and various flavones in sers. *Violaceiflorum* and *Pacificum* that are absent in ser. *Androceras*. The major chemical differences between sers. *Violaceiflorum* and *Pacificum* are flavones with chrysoeriol type B-rings in ser. *Pacificum* and the presence of 8-oxygenated flavonols in ser. *Violaceiflorum* (Whalen 1978a).

Although Whalen (1979a) revised sect. *Androceras* and included a cladistic analysis based on 14 morphological and chemical traits, to date there have been limited molecular phylogenetic studies of this section. Two species of sect. *Androceras*, *S. rostratum* and *S. citrullifolium*, were included in molecular phylogenies of the entire Leptostemonum clade and were strongly supported as sister taxa (Levin et al. 2006; Bohs et al. 2007). These studies place sect. *Androceras* sister to sect. *Crinitum* Child with moderate support (84% bootstrap and 1.0 posterior probability in Levin et al. 2006). This relationship had not previously been proposed due to the fact that sect. *Crinitum* is a South American group of large shrubs and trees with fruits that may reach 10 cm in diameter and large flowers that are not heterantherous. A close relationship between sect. *Androceras* and *S. sisymbriifolium* of sect. *Cryptocarpum* Dunal has been proposed in the past due to their similar leaves, inflorescences, and accrescent calyces (Dunal 1813, 1852; Walpers 1844; Danert 1970; Whalen 1979a; Lester et al. 1999). Both Weese and Bohs (2007) and Bohs et al. (2007) have found that *S. sisymbriifolium* is sister to a clade composed of sect. *Androceras* and sect. *Crinitum*. Whalen (1979a) favored sect. *Nycterium* (Venten.) Walp. as the sister group to sect. *Androceras* based on morphological similarities, but molecular studies unequivocally place the members of sect. *Nycterium* quite distant from sect. *Androceras* (Levin et al. 2006; Bohs et al. 2007; Weese and Bohs 2007). While these studies provide hypotheses about relationships between sect. *Androceras* and other *Solanum* sections, they did not extensively sample from within the section.

In this paper we use molecular phylogenetic methods to 1) test the monophyly of sect. *Androceras* as currently circumscribed, 2) examine the phylogenetic relationships of sect. *Androceras* with closely related members of the Leptostemonum clade, 3) test the monophyly of Whalen's (1979a) series and species within sect. *Androceras*, and 4) examine selected species-level relationships to test hypotheses of character evolution and speciation proposed by Whalen (1979a).

MATERIALS AND METHODS

Taxon Sampling—Eleven of the 12 species and all 10 varieties in sect. *Androceras* sensu Whalen (1979a) were sampled for this study (Table 1). We were unable to obtain high quality genomic DNA for *Solanum leucandrum*, which is known only from the type locality in Puebla, Mexico, due to a lack of available herbarium material. Specimens were determined using keys found in Whalen (1979a), with almost half of the specimens determined by the late Michael D. Whalen himself (indicated with asterisks in Appendix 1). We also included six members of sect. *Crinitum* as well as *S. sisymbriifolium*, both shown by previous molecular studies to be closely related to sect. *Androceras* (Levin et al. 2006; Bohs et al. 2007). Five other more distantly related species from the Acanthophora and Bahamense clades of the Leptostemonum clade were included to ensure sufficient outgroup sampling, and the tree was rooted using *S. betaceum*, an even more distantly related *Solanum* from outside the Leptostemonum clade. The final data set included 43 accessions, representing 11 named species of sect. *Androceras* as well as 12 outgroup species. All taxa, along with voucher information and GenBank accession numbers, are listed in Appendix 1.

DNA Extraction, Amplification, and Sequencing—Total genomic DNA was extracted from fresh, silica gel-dried, or herbarium material using the DNeasy plant mini extraction kit (Qiagen, Inc., Valencia, California). Amplification for each gene region followed standard procedures described in Taberlet et al. (1991), Bohs and Olmstead (2001), and Bohs (2004) for the *trnT-L* and *trnL-F* intergeneric spacer regions; Levin et al. (2005) for *waxy*; and Levin et al. (2006) for ITS. The ITS region was amplified as a single fragment using primers ITSleu1 (Bohs and Olmstead 2001) and ITS4 (White et al. 1990) using PCR conditions described in Bohs and Olmstead (2001). When possible, *trnT-F* and *waxy* were amplified as single fragments using primers a and f for *trnT-F* (Taberlet et al. 1991) and primers waxyF and waxy2R for *waxy* (Levin et al. 2005). Amplification conditions for *trnT-F* followed Bohs and Olmstead (2001); conditions for *waxy* followed Levin et al. (2005). When necessary, overlapping fragments were amplified and assembled, using primers a with d, and c with f to amplify *trnT-F*, and primers waxyF with 1171R, and 1058F with 2R to amplify *waxy*. Specimens not amplifying for *waxy* were amplified in

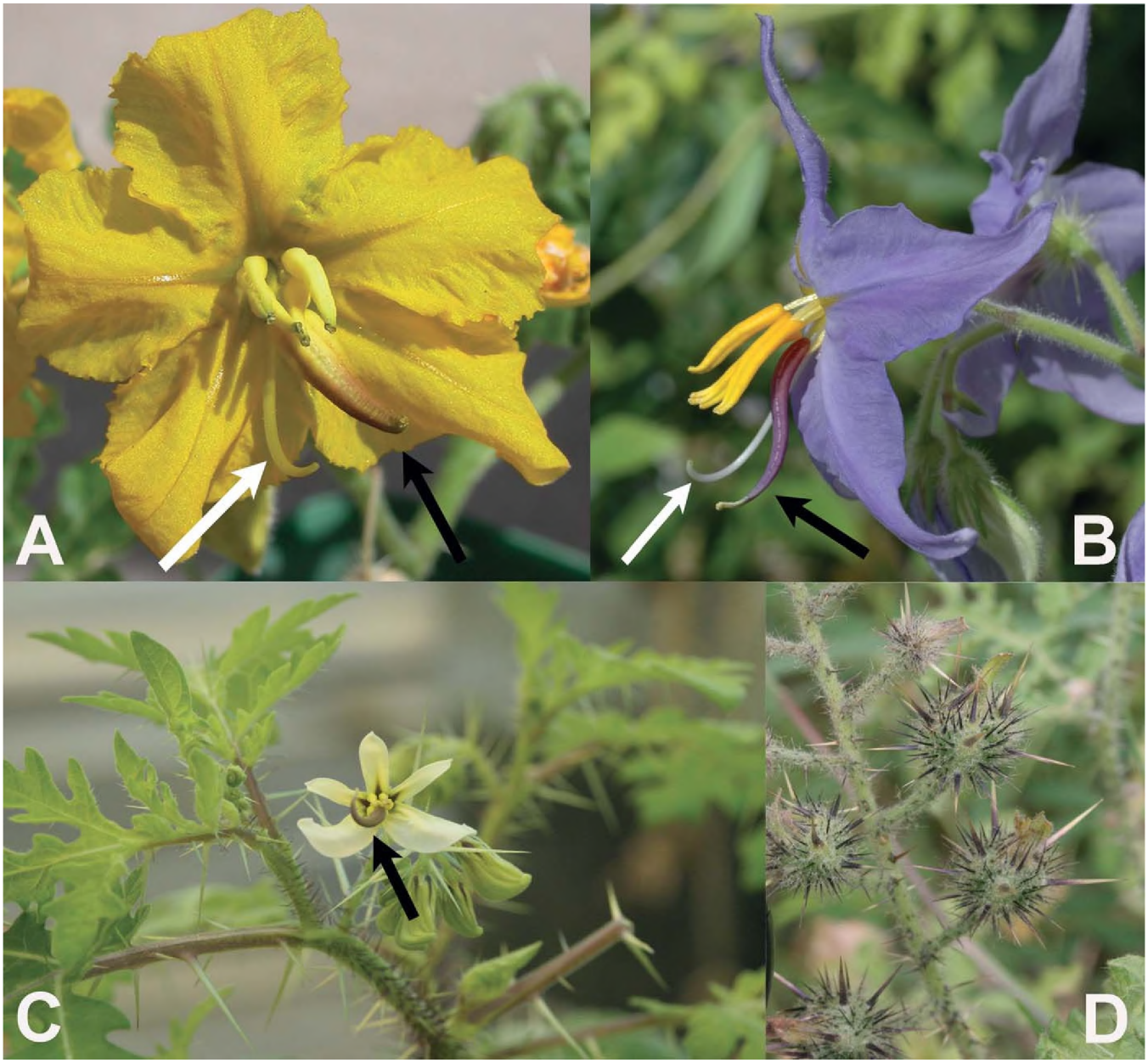

FIG. 1. Representatives of *Solanum* sect. *Androceras*. White arrows indicate the style and black arrows indicate the enlarged lower anther. A. *S. rostratum* of Whalen's ser. *Androceras* and our Rostratum clade. B. *S. citrullifolium* var. *citrullifolium* of Whalen's ser. *Violaceiflorum* and our Setigeroid clade. C. *S. grayii* var. *grandiflorum* of Whalen's ser. *Pacificum* and our Pacificum clade. D. Typical fruits of sect. *Androceras* from *S. rostratum*. Photos C, D courtesy of M. Vallejo-Marin.

even smaller fragments using primers waxyF and the newly developed EX4R (5′-CACAACCTGAACCTAAG-3′) for the first fragment, the new primer EX4F (5′-CTATGGCCCCAAAGCTGGAC-3′) and 1171R for the second fragment, primers 1058F and 3′N (Peralta and Spooner 2001) for the third fragment, and primers 3′F (Miller et al. 1999) and 2R for the final fragment.

Amplification products were cleaned using the Promega Wizard SV PCR Clean-Up System (Promega Corporation, Madison, Wisconsin). The University of Utah DNA Sequencing Core Facility performed sequencing on an ABI automated sequencer. Sequences were edited in Sequencher (Gene Codes Corp., Ann Arbor, Michigan) and all new sequences were submitted to GenBank.

Morphological Data—The data matrix presented in Whalen (1979a; Table 6), representing 11 morphological, two chemical, and one isozyme character for species in sect. *Androceras* was added to the combined molecular data matrix with characters for outgroup species coded as missing data.

Sequence Alignment and Analysis—Sequence alignment for all gene regions was straightforward and performed visually using Se-Al (Rambaut 1996). The aligned datasets and representative phylogenetic trees are available in TreeBASE (study number S2642).

Parsimony Analyses—Maximum parsimony (MP) analyses were performed on each dataset separately and on the combined dataset both with and without morphological data using PAUP*4.0b10 (Swofford 2002). All characters were weighted equally in analyses that implemented tree bisection reconnection (TBR) branch swapping with 1,000 heuristic random addition replicates, each limited to 1,000,000 swaps per replicate. Gaps were treated as missing data. Bootstrapping (BS; Felsenstein 1985) was used to evaluate branch support with 1,000 random addition replicates and TBR branch swapping limited to 1,000,000 swaps per replicate. Datasets were further analyzed using TNT (Goloboff et al. 2008) to search for shorter trees than were obtained in standard PAUP analyses. Congruence of the datasets was tested using partition homogeneity tests (incongruence length difference test [ILD]; Farris et al. 1994, 1995)

implemented in PAUP*. One thousand heuristic partition homogeneity replicates were completed, each with 10 random addition sequence replicates, TBR branch-swapping, MulTrees off, and gaps treated as missing data.

Bayesian Analyses—Prior to Bayesian analyses (BI), a general model of nucleotide evolution was selected for each of the separate and combined datasets using the AIC criterion identified in Modeltest 3.7 (Posada and Crandall 1998). MrBayes 3.1 (Huelsenbeck and Ronquist 2001) was used to analyze the individual and combined datasets. For each analysis 20 replicates were run of four Markov chains, each initiated from a random tree and sampled every 1,000 generations using the stop rule to stop the analysis when standard deviations between the runs reached 0.01. All parameters from each analysis were visualized graphically and the samples obtained prior to achieving stationarity were discarded as a burn-in.

Constraint Analyses—Constraint trees were constructed in MacClade 4 (Maddison and Maddison 2000) to constrain 1) each of Whalen's (1979a) series as monophyletic, 2) only the taxa in ser. *Androceras* as monophyletic, 3) only the taxa in ser. *Violaceiflorum* as monophyletic, and 4) the yellow-flowered taxa as monophyletic. Parsimony analyses were performed with the constraint enforced using TBR branch swapping with 1,000 heuristic random addition replicates, each limited to 1,000,000 swaps per replicate. These trees were then compared with the most parsimonious trees using the Templeton test (Templeton 1983; Prager and Wilson 1988).

Results

Phylogenetic Analyses—Descriptive statistics for the molecular datasets and phylogenetic analyses for the 43 accessions are given in Table 2. Missing data comprised 0.00087% of the combined data matrix (149 bases from a total of 171,907). For the individual datasets, the *trnT-F* region yielded the least resolved phylogeny in both MP and BI analyses. The *waxy* data produced the most resolved trees with the highest number of strongly supported ingroup nodes (Table 2). In general, the parsimony strict consensus and BI majority rule consensus trees from the combined dataset differed only in the degree of resolution, with BI tree topologies more resolved than parsimony trees (Table 2). Clades with low posterior probabilities (PP) in BI analyses were often collapsed in MP strict consensus trees (individual trees not shown).

More nodes were strongly supported by combining the three datasets than were obtained in any of the separate analyses (Table 2; Fig. 2). Inclusion of morphological data did not affect either the topology or resolution of the phylogeny compared to the combined molecular dataset analyzed alone. The only differences between these and the strictly molecular trees were slight differences in support values for a few nodes.

Topological Conflicts—According to the results of the ILD tests, the three data partitions in the combined data set were found to be incongruent ($p = 0.033$), so pairwise ILD tests were run. The nuclear datasets (ITS and *waxy*) were found to be incongruent ($p = 0.01$) as were the *waxy* and *trnT-F* datasets ($p = 0.01$). The only congruent datasets were ITS and *trnT-F* ($p = 0.071$). The incongruence of the datasets is likely due to the disparity in the size and substitution rates of the different datasets (Dolphin et al. 2000; Barker and Lutzoni 2002; Darlu and Lecointre 2002). However, with few exceptions, each DNA sequence region consistently identified the same major, well-supported clades comprising identical species groups, but relationships among clades were often not strongly supported (BS values < 90%), or were unresolved, and thus cannot be considered conflicting under Wiens' (1998) criteria. The BI analysis gave more conflicting nodes (cutoff at < 0.95 PP), but posterior probabilities are known to be inflated relative to bootstrap values (Cummings et al. 2003; Erixon et al. 2003; Simmons et al. 2004). Our discussion will be focused on the topology of the BI majority rule and MP strict consensus trees based on combined molecular data (Fig. 2).

Phylogenetic Relationships—Sectional Relationships and Monophyly of Section *Androceras*—All data sets strongly support the monophyly of sect. *Androceras* as circumscribed by Whalen (1979a, 1984; 100% BS, 1.0 PP in ITS, *waxy* and combined gene trees and 99% BS, 1.0 PP in *trnT-F*).

Although not supported in the single-gene analyses, the combined dataset supports sect. *Crinitum* as sister to sect. *Androceras* (88% BS, 1.0 PP), with *Solanum sisymbriifolium* sister to the clade composed of sects. *Androceras* and *Crinitum* (85% BS, 1.0 PP).

Monophyly of the Series Within Section *Androceras*—Of the three series identified by Whalen (1979a), our phylogeny supports only ser. *Pacificum* as a monophyletic group, termed the Pacificum clade in Fig. 2. This relationship is supported in the individual ITS (87% BS, 1.0 PP) and *waxy* datasets (98% BS, 1.0 PP) but not in the *trnT-F* dataset; the combined dataset resolves this group with 100% BS and 1.0 PP. Three of the five species of ser. *Androceras* form a moderately to strongly supported Rostratum clade composed of *S. rostratum*, *S. fructotecto*, and *S. angustifolium* in the *waxy* only (82% BS, 1.0 PP) and combined trees (94% BS, 1.0 PP). *Solanum johnstonii* of ser. *Androceras* is unplaced in the ITS, *trnT-F*, and combined analyses; the *waxy* only analysis places this species as sister to the Pacificum clade with moderate support (86% BS, 1.0 PP). The final member of Whalen's ser. *Androceras*, *S. tribulosum*, is moderately supported (82% BS, 1.0 PP) as sister to a large clade of species, placed by Whalen (1979a) in ser. *Violaceiflorum*, in the combined analyses, but this relationship is not recovered in any of the individual analyses. Whalen's ser. *Violaceiflorum* is clearly polyphyletic, with a large clade composed of *S. heterodoxum* var. *setigeroides*, *S. citrullifolium* vars. *citrullifolium* and *setigerum*, and *S. davisense* forming a monophyletic group, here termed the Setigeroid clade, in the *waxy* only (99% BS, 1.0 PP) and combined analyses (92% BS, 1.0 PP; Fig. 2). The remainder of the taxa belonging to Whalen's ser. *Violaceiflorum*, including *S. tenuipes*, *S. citrullifolium* var. *knoblichii*, and *S. heterodoxum* vars. *heterodoxum* and *novomexicanum* form a grade at the base of the Androceras clade in the combined analyses. "Elder 46", a potentially undescribed

Table 2. Descriptive statistics for the datasets analyzed. Strongly supported nodes for parsimony indicate those with ≥ 90% BS; Bayesian strongly supported nodes are those with ≥ 0.95 PP.

Data Partition	Aligned Sequence Length	Number of Parsimony Informative Characters	Number of MP Trees	Tree Length	CI	RI	Number of Strongly Supported Nodes Parsimony (ingroup nodes)	Model Selected	Number of Strongly Supported Nodes Bayesian (ingroup nodes)
ITS	666	121	13,691	431	0.608	0.783	11 (6)	GTR + I + G	21 (15)
waxy	1,731	165	48	428	0.844	0.895	17 (12)	GTR + G	32 (24)
trnT-F	2,088	65	52,750	188	0.910	0.895	6 (3)	GTR + I + G	13 (8)
Combined	4,485	340	10	1,085	0.733	0.829	18 (12)	GTR	36 (24)
Combined + Morphological	4,499	354	6	1,127	0.726	0.828	16 (12)	GTR	38 (27)

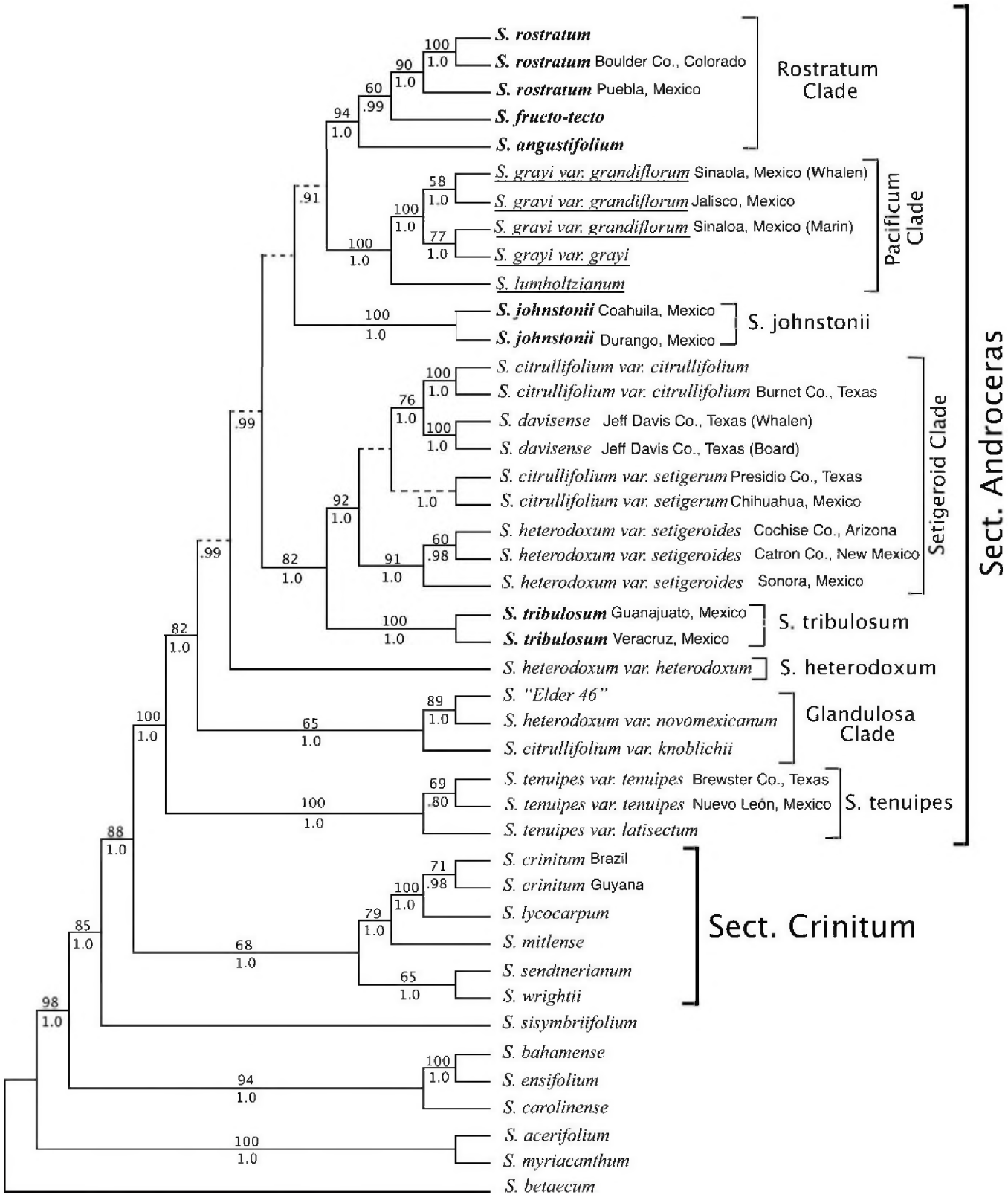

FIG. 2. 50% majority rule tree from the Bayesian analysis of the combined dataset. Numbers above branches are bootstrap values over 50%; numbers below branches are posterior probabilities from Bayesian analysis. Branches that collapse in the parsimony strict consensus tree but are present in the Bayesian majority rule tree are shown as dashed lines. Species of sect. *Androceras* placed by Whalen (1979a) in ser. *Androceras* are in bold italics, in ser. *Pacificum* are underlined, and in ser. *Violaceiflorum* are in nonbold italics. *Solanum* "Elder 46" was not placed in any of Whalen's (1979a) series; see text for discussion. The clades discussed in the text are labeled.

species, is strongly supported as sister to *S. heterodoxum* var. *novomexicanum* in the ITS only (85% BS, 0.98 PP), *waxy* only (87% BS, 1.0 PP) and combined analyses (89% BS, 1.0 PP), and, along with *S. citrullifolium* var. *knoblichii*, comprises a monophyletic group in the *waxy* only (100% BS, 1.0 PP) and combined analyses (65% BS, 1.0 PP), here termed the Glandulosa clade.

Species- and Infraspecific-Level Monophyly—Species-level monophyly was examined in a number of taxa with multiple accessions sequenced in the phylogeny. In the cases of *S. rostratum*, *S. grayi*, *S. johnstonii*, *S. davisense*, *S. tribulosum*, and *S. tenuipes*, all accessions of the same species formed monophyletic groups with strong support in the combined trees. Furthermore, the multiple accessions sequenced of *S. citrullifolium* var. *citrullifolium* and *S. heterodoxum* var. *setigeroides* each emerged as monophyletic in all combined analyses, but *S. citrullifolium* var. *setigerum* is paraphyletic in the combined MP strict consensus tree. However, *S. citrullifolium*, *S. heterodoxum*, and *S. grayi* var. *grandiflorum* were not supported as monophyletic, as multiple accessions of these taxa did not group together in the combined analyses.

Constraint Analyses—Constraining all of Whalen's series to be monophyletic resulted in trees significantly different than the most parsimonious tree from the combined dataset (Templeton's test $p = 0.0001$). When constraining sers. *Androceras* and *Violaceifolium* individually, the trees were also significantly different than the most parsimonious tree from the combined dataset ($p = 0.0455$ and 0.0477, respectively). Trees constraining all of the yellow-flowered taxa (i.e. species of ser. *Androceras* minus *S. tribulosum*) to monophyly were not significantly different than nonconstrained trees (Templeton's test $p = 0.6698$).

Discussion

Sectional Relationships and Monophyly of Section Androceras—Despite the various hypotheses regarding the sister group to sect. *Androceras*, our data support previous molecular studies in finding sect. *Crinitum* as sister to sect. *Androceras* (Levin et al. 2006; Weese and Bohs 2007). These groups are morphologically distinctive and this relationship merits further study. *Solanum sisymbriifolium* is sister to a clade composed of sect. *Androceras* and sect. *Crinitum* despite the fact that *S. sisymbriifolium* and sect. *Androceras* share highly divided leaves and strongly accrescent calyces, characters not found in sect. *Crinitum*. Lester et al. (1999) also found the seeds of sects. *Androceras* and *Cryptocarpum*, to which *S. sisymbriifolium* belongs, to be remarkably similar. We were not able to sample other members of sect. *Cryptocarpum* but further sampling might possibly place this group sister to sect. *Androceras*.

All three data sets strongly support the monophyly of sect. *Androceras* as circumscribed by Whalen (1979a, 1984). This molecular evidence, combined with unique morphological traits found in the leaves, flowers, and fruits, its distinctive flavonoid chemistry, and geographical distribution, leave little doubt that *Solanum* sect. *Androceras* is a monophyletic group.

Character Evolution and Monophyly of Whalen's Series in sect. Androceras—Whalen's (1979a) three series within sect. *Androceras* were distinguished by trichome, flower, and seed morphology as well as flavonoid chemistry and geographical distribution (Table 1). Whalen (1979a) circumscribed these series as natural phyletic groups; however, they were not defined in strict monophyletic terms (see paraphyly of sers. *Androceras* and *Violaceiflorum* in Fig. 15 in Whalen 1979a). It is clear in examining his matrix of morphological characters (Table 6 and Fig. 15 in Whalen 1979a), that many are homoplasious or autapomorphic. Additionally, the assessment of ancestral and derived characters as well as coding of characters are based on the author's interpretations (see secondarily lost characters in Table 5 in Whalen 1979a) and could be differently interpreted by other taxonomists. Given this and the fact that our combined molecular dataset contains 340 parsimony informative characters, it is not surprising that the addition of the 14 characters from Whalen's (1979a) dataset does not change the topology or resolution of the phylogeny (results not shown). The few synapomorphic characters in Whalen's (1979a) character matrix show support for ser. *Pacificum*, the only one of the three series that emerges as a monophyletic group in our molecular trees. Characters unique to this series include white, deeply stellate corollas, radially wrinkled seeds, and a geographical center of distribution on the Pacific slope of the Sierra Madre Occidental on the west coast of Mexico. Apparently these characters arose once in the Pacificum clade, although confirmation of this awaits sampling of the third member of ser. *Pacificum*, *S. leucandrum*.

Neither ser. *Androceras* nor *Violaceifolium* is supported as monophyletic in the molecular analyses. These series were paraphyletic in Whalen's (1979a) cladistic analysis and the nonmolecular characters that supported these groups are likely convergent. For instance, ser. *Androceras* was characterized by Whalen (1979a) as having stellate or multangulate cauline hairs and yellow corollas, lacking flavonoid compounds found in the other two series, and a distribution centered in the central Mexican highlands around Mexico City. These characters are found in species of the Rostratum clade (Fig. 2), but also in *S. johnstonii*, which does not form a part of this clade. Conversely, Whalen (1979a) placed *S. tribulosum* into ser. *Androceras* despite its pale blue or white corollas. Support and resolution along the backbone of the tree obtained here is weak or lacking, precluding firm conclusions about character evolution in sect. *Androceras* based on the most parsimonious trees. However, constraining all three series each to be monophyletic as well as constraining the taxa of Whalen's sers. *Androceras* and *Violaceifolium* individually to be monophyletic resulted in trees significantly different than the most parsimonious trees from the combined dataset. This further indicates that these two series are likely nonmonophyletic and that the characters that Whalen proposed to diagnose them have evolved multiple times. On the other hand, when all yellow-flowered taxa (i.e. species of ser. *Androceras* minus *S. tribulosum*) were constrained to monophyly, the constrained trees were not significantly different than nonconstrained trees. Therefore, the hypothesis of a single origin of yellow corollas within sect. *Androceras* cannot be rejected.

According to Whalen (1979a), nine of the species of sect. *Androceras* are taprooted annual herbs with wide edaphic tolerances. *Solanum johnstonii*, *S. tenuipes*, and *S. tribulosum*, however, are calciphilic herbaceous perennials. Judging from their widely separated positions on the molecular trees, it appears that the latter traits evolved independently in the three species.

Biogeographical Relationships—Based on his interpretation of cladistic relationships in sect. *Androceras*, Whalen

(1979a) considered ser. *Androceras* to be plesiomorphic within the section, implying an origin for the section in the central Mexican highlands (Whalen 1979a, 1983). However, the molecular phylogenies place *S. tenuipes*, included in ser. *Violaceifolium* by Whalen (1979a), as sister to the remainder of sect. *Androceras* with good support (82% BS, 1.0 PP). *Solanum tenuipes* occurs in the northern Chihuahua Desert near the Texas-Mexico border, pointing to a more northerly origin for the section. In the BI trees, the Glandulosa clade is in turn sister to the remainder of the species (Fig. 2). Species of this clade are also found in the northern Chihuahua Desert and range into the southwestern U. S. A., consistent with a northern origin. However, this latter relationship is poorly supported and collapses in the MP strict consensus trees. Nonetheless, molecular evidence refutes Whalen's (1979a, 1983) hypothesis of a central to southern Mexican origin for sect. *Androceras*.

Clades Within sect. Androceras—ROSTRATUM CLADE—The Rostratum clade contains *S. rostratum*, *S. fructo-tecto*, and *S. angustifolium*, three of the five species placed by Whalen (1979a) in ser. *Androceras*. *Solanum rostratum* is a widely introduced weed and is common in the central and western U. S. A., but Whalen (1979a) considered central Mexico to be its area of origin due to the high level of morphological variability in this region and because many of the sister taxa proposed by Whalen (1979a) occur there. Our phylogeny samples accessions from both the U. S. A. and Mexico and all form a strongly supported group. Combined with many morphological characters, there is little doubt that, although it is the most widespread species in the section, *S. rostratum* is a monophyletic and distinct species. The other members of the Rostratum clade have more restricted distributions: *S. fructo-tecto* is found in the vicinity of Mexico City and Ciudad Durango, and *S. angustifolium* is found from southern Mexico through Honduras. Although *S. fructo-tecto* is vegetatively similar to *S. rostratum*, Whalen did not encounter hybrids or collections intermediate between the two species in reproductive characteristics. Therefore, he states that the overlap in vegetative characteristics between the species probably represents natural variation. Whalen (1979a) considered *S. angustifolium* to be closely related to *S. rostratum* but also called it a bridging taxon between his sers. *Androceras* and *Violaceiflorum*. Our phylogeny indicates that, despite sharing trichome and flavonoid characters with species in Whalen's ser. *Violaceiflorum*, *S. angustifolium* is in fact closely related to *S. rostratum*.

PACIFICUM CLADE—The Pacificum clade is found in western Mexico along the Pacific slope of the Sierra Madre Occidental and inland in central Mexico. This clade comprises two of the three species placed by Whalen (1979a) in ser. *Pacificum*; *S. leucandrum*, the third, was not sampled. *Solanum grayi* has been divided into two varieties based on flower size. The small-flowered form is known as *S. grayi* var. *grayi*, whereas the large-flowered plants are segregated as var. *grandiflorum*. Our phylogeny sampled species from throughout the range of *S. grayi* and did not consistently separate these varieties. These varieties seem to have arisen from character displacement in areas where *S. grayi* occurs sympatrically with its purported sister species *S. lumholtzianum*. Whalen (1978b) showed that *S. lumholtzianum* and *S. grayi* have similar sized flowers over their distinctive ranges, but show strong character displacement where their ranges overlap in Sonora and northern Sinaloa, with the flowers of *S. grayi* much smaller there than in other parts of its range. *Solanum grayi* and *S. lumholtzianum* were shown to successfully hybridize in experimental crosses, but Whalen (1978b, 1979a) posits mechanical isolation via character displacement of floral traits in areas where the two species overlap, indicating that in nature they would not share the same pollinators and would effectively be reproductively isolated. Although our phylogenetic data suggest that the varietal distinctions in *S. grayi* might not be warranted, additional sampling from this species is needed to examine this question. The final member of Whalen's ser. *Pacificum*, *S. leucandrum*, is a rarely collected species and thus material was not available for this study. It is endemic to western Puebla and is morphologically similar to *S. grayi*, thus would likely be included in the Pacificum clade.

SETIGEROID CLADE—The Setigeroid clade is strongly supported in our phylogeny and contains *S. davisense*, *S. citrullifolium* vars. *citrullifolium* and *setigerum*, and *S. heterodoxum* var. *setigeroides*. These species all occur in the southwestern U. S. A. and the area along the Texas-Mexico border. Our phylogeny shows that *S. davisense* is closely related to *S. citrullifolium* var. *citrulllifolium* (76% BS, 1.0 PP), a result supported by allozyme data from Whalen (1979b). *Solanum davisense* is distinct from the other species of the Setigeroid clade due a more erect habit, acutely lobed leaves, smaller flowers, and smooth unridged seeds as well as chemical differences (Whalen 1979a). Divergence of *S. davisense* and *S. citrullifolium* was likely due to the slight geographical separation of *S. davisense* at the margin of the range of *S. citrullifolium* var. *citrulllifolium* (Whalen 1979a, 1979b). *Solanum citrullifolium* vars. *setigerum* and *citrullifolium* do not form a monophyletic group in either the MP or BI combined analysis. Monophyly of *S. citrullifolium* var. *setigerum* itself is not supported in the MP strict consensus tree, yet it receives strong support (1.0 PP) in the BI 50% majority rule tree. Therefore, it is unclear whether the two varieties should be recognized as taxonomically distinct entities. As indicated by the common varietal name *setiger-* (Latin for "bristly"), *S. citrullifolium* var. *setigerum* and *S. heterodoxum* var. *setigeroides* share morphological similarities and have also been found to have a history of hybridization (Whalen 1979a). This, combined with the phylogenetic relatedness of these taxa, warrants a more detailed taxonomic investigation of these species and varieties to determine the relationship and specific delimitations of members of the Setigeroid clade.

GLANDULOSA CLADE—The Glandulosa clade presents interesting taxonomic and biogeographic problems. This clade contains *S. citrullifolium* var. *knoblichii*, *S. heterodoxum* var. *novomexicanum* and an unidentified species here called "Elder 46" based on the collector and collection number. *Solanum citrullifolium* var. *knoblichii* morphologically resembles var. *citrullifolium* but is restricted to western Chihuahua state in Mexico. It has longer hairs and more spreading fruit pedicels than the other varieties of *S. citrullifolium* but, due to a lack of collections, other morphological differences are not apparent. It is distantly related to its conspecifics, which occur in the Setigeroid clade (see above), and deserves further collection and taxonomic study. *Solanum heterodoxum* var. *novomexicanum* was given specific status [as *Androcera novomexicana* (Bartlett) Wooten & Standl.] by Wooten and Standley (1913). Despite the large geographic separation between *S. heterodoxum* var. *heterodoxum* from the area around Mexico City and var. *novomexicanum* from New Mexico, Whalen (1979a) felt that these varieties resembled each other except for the more stellate corollas in the latter variety. The geographically close *S. heterodoxum* var. *setigeroides* occurs in adjacent areas of New Mexico, Arizona, and the Texas-Mexico border. This variety

is distinct morphologically, with densely prickly stems and much finer spines than the other varieties of *S. heterodoxum*. Given the distinct morphological traits and the phylogenetic distance between *S. heterodoxum* var. *novomexicanum* and the other varieties, the specific classification of Wooten and Standley (1913) should be reconsidered. The final member of this clade, "Elder 46," is a collection from Jeff Davis County, Texas. This specimen has previously been identified as *S. grayi* var. *grandiflorum*, *S. davisense*, and *S. heterodoxum* but does not fit any of those species concepts. Whalen did not examine this specimen, and use of his key and comparison to specimens he annotated does not result in a satisfactory determination. Since it appears that many of the species in the section are restricted endemics, it is possible that this collection represents an undescribed species.

The three species in the Glandulosa clade share some morphological characteristics including a diminutive weedy annual habit, violet or occasionally white flowers, and simple, often glandular hairs. Whalen (1979a) notes that the flavonoid profile for *S. heterodoxum* var. *novomexicanum* is identical to that of var. *heterodoxum*; however, the other members of the Glandulosa clade have not been sampled. The species in the Glandulosa clade have geographic ranges that do not appear to overlap, with "Elder 46" occurring in Jeff Davis County, Texas, *S. heterodoxum* var. *novomexicanum* occurring in north central New Mexico, and *S. citrullifolium* var. *knoblichii* restricted to Chihuahua, Mexico. Further systematic study and field collections will help to clarify the number of distinct taxa represented within this clade.

Solanum johnstonii—The two accessions of *S. johnstonii* emerge as a monophyletic group. This species has a very restricted range in the Durango state of north-central Mexico and has often been identified as *S. rostratum*. However, Whalen (1979a) cites many morphological differences as well as reproductive isolation as evidence that *S. johnstonii* and *S. rostratum* are distinct species. Our phylogeny supports this separation, but there is little support for the relationship of *S. johnstonii* with any of the other clades within sect. *Androceras*.

Solanum tribulosum—*Solanum tribulosum* shares purple flower color with members of the Whalen's ser. *Violaceifolium*, but he placed it in ser. *Androceras* due to geographical distribution, chemical characteristics (notably a lack of 8-hydroxyflavonoids and various flavones that are found in ser. *Violaceifolium*) and morphological features such as stellate corollas and smooth seeds. Results from the combined analyses indicate that *S. tribulosum* is more closely related to the other purple-flowered taxa here placed in the Setigeroid clade than to the species of Whalen's ser. *Androceras*, which include *S. rostratum*, *S. fructo-tecto*, and *S. angustifolium* (Rostratum clade) as well as *S. johnstonii*.

Solanum heterodoxum var. *heterodoxum*—The position of *S. heterodoxum* var. *heterodoxum* within the section is unresolved and it is not placed with either of the other *S. heterodoxum* varieties. This isolated phylogenetic position mirrors its geographical disjunction; *Solanum heterodoxum* vars. *setigeroides* and *novomexicanum* occur in the southwestern U. S. A., and northern Mexico, whereas var. *heterodoxum* is greatly disjunct in central Mexico. *Solanum heterodoxum* var. *heterodoxum* has less prickly stems with much stouter prickles than those of var. *setigeroides* and flowers with much more interpetalar tissue than those of var. *novomexicanum*. These differences, along with the phylogenetic results, indicate that *S. heterodoxum* as currently defined is almost certainly not monophyletic.

Solanum tenuipes—The two varieties of *S. tenuipes* are placed together as a strongly supported monophyletic group sister to all the other taxa of sect. *Androceras*. This species is found along the Texas-Mexico border and is divided into var. *tenuipes* and var. *latisectum* based on geography, leaf dissection, and seed size. Whalen (1979a) notes intermediates between these varieties and our phylogeny gives only weak support to grouping the two accessions of var. *tenuipes* (69% BS, 0.80 PP). Whalen (1979a) considered *S. tenuipes* to be derived within the section, making the placement of *S. tenuipes* at the base of sect. *Androceras* unexpected and worthy of further investigation.

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APPENDIX 1. Summary of species, collection location, vouchers, and GenBank accession numbers for taxa used in this study provided in the order ITS, *waxy*, and *trnT-F*. Asterisks indicate specimens identified by M. D. Whalen. NIJ – cultivated at Radboud University, Nijmegen, The Netherlands.

S. acerifolium Dunal - Costa Rica, *Bohs 2714* (UT); AY561261, AY562949, AY266149. ***S. angustifolium*** Mill. - Oaxaca, Mexico, *Whalen 2* (LL)*; GQ143645, GQ143677, GQ149729. ***S. bahamense*** L. - NIJ 944750187, *Bohs 2936* (UT); AY996487, AY996386, GQ149730. ***S. betaceum*** Cav. – Bolivia, *Bohs 2468* (UT); AF244713, AY996387, DQ180426. ***S. carolinense*** L. – U. S. A., *Cipollini s. n.* (UT); AY996491, AY996392, DQ180476. ***S. citrullifolium* var. *citrullifolium*** - Burnet Co., Texas, *Urbatsch 4834* (NY); GQ143647, GQ143679, GQ149732. NIJ 894750197, *Bohs 3452* (UT); GQ143646, GQ143678, GQ149731. ***S. citrullifolium* var. *knoblichii*** Whalen - Chihuahua, Mexico, *Lebgue 3266* (NY); GQ143648, GQ143680, GQ149733. ***S. citrullifolium* var. *setigerum*** Bartlett - Chihuahua, Mexico, *Whalen 365* (LL)*; GQ143650, GQ143682, GQ149735. Presidio Co., Texas, *Turner 24-245* (TEX); GQ143649, GQ143681, GQ149734. ***S. crinitum*** Lam. – Brazil, *Agra et al. 7028* (JPB); GQ143651, GQ143683, GQ149736. Guyana, *Stern 255* (UT); GQ143652, GQ143684, GQ149737. ***S. davisense*** Whalen - North population Jeff Davis Co., Texas, *Board s. n.* (NY); GQ143654, GQ143686, GQ149739. South population Jeff Davis Co., Texas, *Whalen 216* (LL)*; GQ143653, GQ143685, GQ149738. ***S. "Elder 46"*** - Jeff Davis Co., Texas, *Elder 46* (TEX); GQ143655, GQ143687, GQ149740. ***S. ensifolium*** O. E. Schulz - Puerto Rico, *Bohs 2461* (UT); AY996506, AY996409, DQ180483. ***S. fructo-tecto*** Cav. - Distrito Federal, Mexico, *Iltis 28607* (NY); GQ143656, GQ143688, GQ149741. ***S. grayi* var. *grandiflorum*** Whalen - Jalisco, Mexico, *Guadalupe Ayala #91-9* (TEX); GQ143658, GQ143691, GQ149743. Sinaloa, Mexico, *Vallejo-Marin 07s195* (MEX); GQ143659, GQ143690, GQ149744. Sinaloa, Mexico, *Whalen 190* (LL)*; GQ143657, GQ143689, GQ149742. ***S. grayi* var. *grayi*** - Sonora, Mexico, *Reina 99-469* (TEX); GQ143660, GQ143692, GQ149745. ***S. heterodoxum* var. *heterodoxum*** - San Luis Potosí, Mexico, *Fryxell 3810* (NY); GQ143661, GQ143693, GQ149746. ***S. heterodoxum* var. *novomexicanum*** Bartlett - San Miguel Co., New Mexico, *Whalen 224* (LL)*; GQ143662, GQ143694, GQ149747. ***S. heterodoxum* var. *setigeroides*** Whalen - Cantron Co., New Mexico, *Shelton 127* (NY); GQ143664, GQ143696, GQ149749. Cochise Co., Arizona, *McGill 6785* (TEX); GQ143663, GQ143695, GQ149748. Sonora, Mexico, *Minckley s. n.* (UT) ; GQ143665, GQ143697, GQ149750. ***S. johnstonii*** Whalen - Coahuila, Mexico, *Villarreal 4404* (TEX); GQ143666, GQ143698, GQ149751. Durango, Mexico, *Villarreal 6246* (TEX); GQ143667, GQ143699, GQ149752. ***S. lunholtzianum*** Bartlett - Sonora, Mexico, *Reina 99-398* (TEX); GQ143668, GQ143700, GQ149753. ***S. lycocarpum*** A. St.-Hil. – Paraguay, *Bohs 3212* (UT); AY996525, AY996435, DQ812107. ***S. mitlense*** Dunal – Mexico, *Whalen & Velasco 825* (BH); AY996530, AY996442, DQ812108. ***S. myriacanthum*** Dunal - NIJ 814750043, *Cipollini 83* (UT); AY561267, AY562960, AY559240. ***S. rostratum*** Dunal - Boulder Co., Colorado (no voucher); AY996550, AY996463, DQ180489. NIJ 934750126, *Cipollini 173* (UT); GQ143670, GQ143702, GQ149755. Puebla, Mexico, *Cipollini 184* (UT); GQ143669, GQ143701, GQ149754. ***S. sendtnerianum*** Van Heurck & Müll. Arg. – Brazil, *Lepsch de Cunha & Wang 310* (MO); GQ143671, GQ143703, GQ149756. ***S. sisymbriifolium*** Lam. – Bolivia, *Cipollini 132* (UT); AY561271, AY562967, AY266235. ***S. tenuipes* var. *latisectum*** Whalen - Chihuahua, Mexico, *Whalen 72* (LL)*; GQ143672, GQ143705, GQ149757. ***S. tenuipes* var. *tenuipes*** - Brewster Co., Texas, *Whalen 218* (LL)*; GQ143673, GQ143706, GQ149758. Nuevo León, Mexico, *Hinton 22874* (TEX); GQ143674, GQ143704, GQ149759. ***S. tribulosum*** Schauer - Guanajuato, Mexico, *Ventura 8236* (TEX); GQ143675, GQ143707, GQ149760. Veracruz, Mexico, *Whalen 18* (LL)*; GQ143676, GQ143708, GQ149761. ***S. wrightii*** Benth. - Costa Rica, *Bohs 2445* (UT); GQ480731, GQ480733, GQ480732.