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An Eight Marker Phylogeny of *Solanum* sect. *Micracantha* (Solanaceae)

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Abstract—The 12 species of *Solanum* sect. *Micracantha* are part of the large subgenus *Leptostemonum* or "spiny solanums," which includes some 350–400 species. Members of the section are found in the Neotropics and are characterized by a combination of characters including a viny, climbing habit using recurved prickles, unbranched inflorescences, deeply divided corollas, and glabrous fruits. Broad-scale phylogenetic relationships within the spiny solanums have been the subject of several recent studies, but many of the individual groups, including sect. *Micracantha*, have received little attention. In this study we infer relationships within sect. *Micracantha* and its placement within the spiny solanums using DNA sequence data from three chloroplast markers, *trnT-F, rpl32-trnL,* and *ndhF-rpl32* and five nuclear markers, ITS, *waxy,* and three COSII markers: At1g44760 (COS4b), At1g50020 (COS5c), and At2g15890 (COS5). Results support the monophyly of sect. *Micracantha* (the Micracantha clade) and its sister relationship with the Bahamense clade of the spiny solanums. Within sect. *Micracantha* it appears that characteristics such as fruit size and habit are more strongly correlated with the habitat in which the species grow than with the phylogeny. The COSII markers had numerous parsimony-informative characters and are similar in their utility to the more commonly used ITS and *waxy* markers. The *trnT-F* chloroplast marker resolved more nodes than the *ndhF-rpl32* and the *rpl32-trnL* markers in parsimony analyses, despite the greater number of parsimony-informative characters in *rpl32-trnL*.

Keywords—COSII markers, *ndhF-rpl32*, *rpl32-trnL*.

The 12 species of *Solanum* sect. *Micracantha* Dunal are members of the large subgenus *Leptostemonum* (Dunal) Bitter (Whalen 1984; Nee 1999; Levin et al. 2006). This subgenus contains the eggplant (*S. melongena* L.) and is commonly referred to as the "spiny solanums" due to the presence of sharp epidermal prickles. *Solanum* sect. *Micracantha* is a group of scandent shrubs or vines found in disturbed areas from southern Florida and the Caribbean through Central America and South America as far south as Bolivia.

The two most recent circumscriptions of sect. *Micracantha* are those of Whalen (1984) and Nee (1999). These classifications referred to the species as the *S. lanceifolium* species group and sect. *Micracantha*, respectively. These two classifications share many species, but Nee's (1999) circumscription is more inclusive (Fig. 1). Whalen's (1984) species group largely correlates with Nee's (1999) subsect. 1, series 1, but also includes *S. leucopogon* and *S. coriaceum* from Nee's subsect. 1, series 3 and 4, respectively. Both authors focused on a combination of characters to circumscribe the group, including a scandent or viny climbing habit using recurved prickles, unbranched inflorescences, deeply divided corollas, and glabrous fruits (Fig. 2). This combination of characters is unique to sect. *Micracantha*; however, species in other sections of *Solanum* have converged on one or more of these traits, which has made delimitation of the section very difficult.

The species of sect. *Micracantha* are found throughout the Neotropics. Just under half are restricted to small geographic areas such as *S. apaporanum,* endemic to the Amazon lowlands in Colombia, Peru, and Brazil, *S. arachnidanthum*, found only in the lowlands of northern Bolivia, and *S. flexicaule*, restricted to the western slope of the Andes in Ecuador. On the northern coast of South America, *S. monachophyllum* occurs on the Guiana Shield and adjacent areas of the Amazon Basin, and *S. asperrimum* is found at mid-elevations in eastern Colombia and northwestern Venezuela. These contrast with the widespread *S. jamaicense*, which ranges from the southern United States and Caribbean to Bolivia. Four species, *S. aturense, S. lanceifolium, S. tampicense* and *S. volubile*, are found in Mexico, Central America, the Caribbean, and reach into northwestern South America while *S. leucopogon* and *S. pedemontanum* are widespread along the eastern slope of the Andes from Colombia to Bolivia.

All members of sect. *Micracantha* are upright or scandent shrubs or vines with recurved prickles that aid in climbing. They are all plants of disturbed areas but habitats vary from riverbanks and swampy areas to disturbed pastures and roadsides to gaps in primary forest. Inflorescences in species of sect. *Micracantha* are unbranched and shorter than those of some other spiny solanum groups, such as sects. *Torva* or *Crinitum.* The corollas in sect. *Micracantha* are deeply stellate with little interpetalar tissue, whereas many other spiny solanums have more shallowly divided corollas with abundant interpetalar tissue. The fruits of sect. *Micracantha* are fleshy berries that range from small (< 10 mm) and thin-walled to larger (> 20 mm) with a leathery pericarp.

Previous molecular studies (Levin et al. 2006; Stern et al. 2011) sampled taxa from sect. *Micracantha* in order to determine its monophyly and place it within the phylogenetic context of the spiny solanums. Levin et al. (2006) sampled S. *adhaerens, S. aturense*, and *S. jamaicense* and determined that they form a well-supported group (the Micracantha clade), which was part of a polytomy with a large number of other spiny solanum clades. Stern et al. (2011) increased sampling to nine species putatively belonging to sect. *Micracantha* and found that they all formed a well-supported clade. However, resolution was poor within the *Micracantha* clade, and it again formed a polytomy with several other spiny solanum groups.

The goals of this study are to generate a molecular phylogenetic hypothesis for species of *Solanum* sect. *Micracantha* using increased taxonomic sampling and additional molecular markers compared to the analyses of Levin et al. (2006) and Stern et al. (2011). Following the convention used by other molecular phylogenetic studies of *Solanum*, results will reference groups using informal clade names (Levin et al. 2006; Stern et al. 2011). This study used eight markers, the plastid *trnT-F*, *ndhf-rpl32*, and *rpl32-trnL*, and the nuclear ITS, *waxy* (or GBSSI), and three conserved orthologous set (COSII) markers. The utility of the *rpl32-trnL* and *ndhF-rpl32* regions for phylogenetic inference was shown by Shaw et al. (2007) for a wide range of angiosperms and has been used

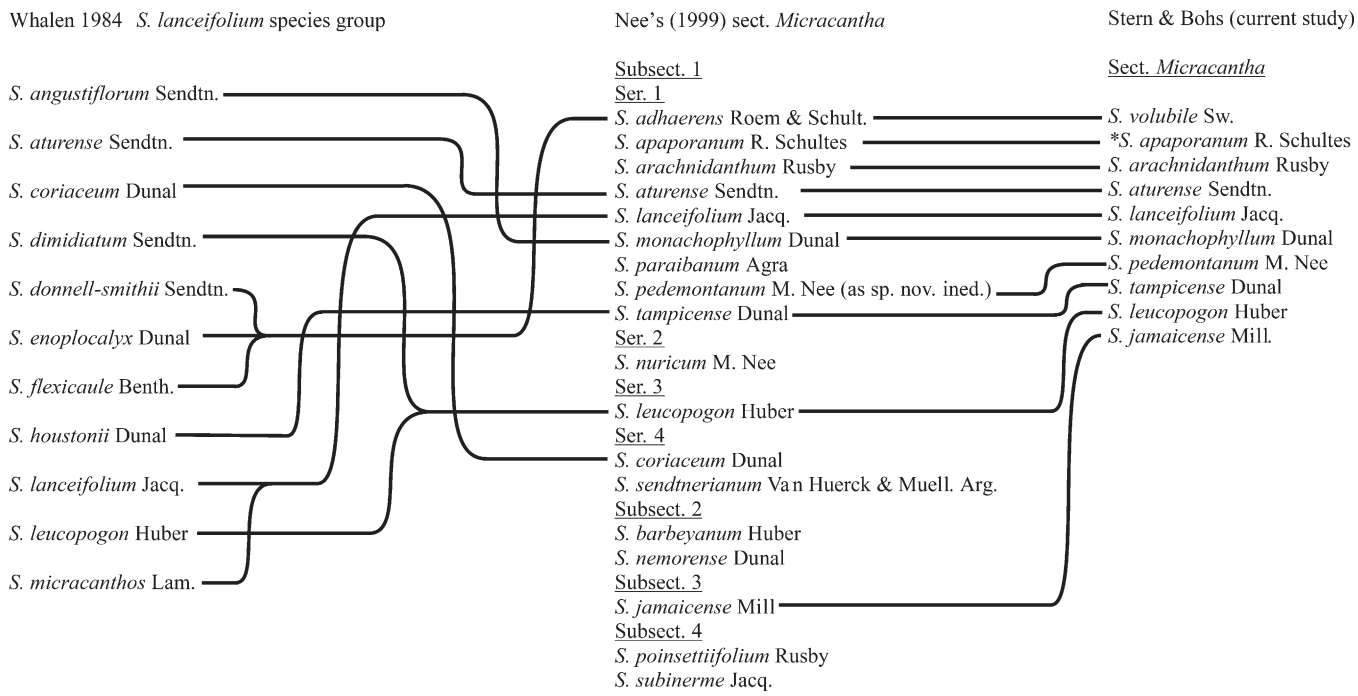

FIG. 1. Flow chart of various circumscriptions of the Micracantha clade as recognized by Whalen (1984), Nee (1999), and the current study. The placement of species not included in the Micracantha clade in the current study but included in Whalen's (1984) and Nee's (1999) circumscriptions is discussed in the text. Asterisks indicate species for which molecular data was not available but whose morphology fits with that of the Micracantha clade.

within the Solanaceae by Miller et al. (2009). The COSII markers are a recently developed set of markers for phylogenetic inference within the Asterid clade (Wu et al. 2006). They are single- or low-copy markers found throughout the nuclear genome that are both intronic and exonic, giving a wide range of variability for clarifying relationships at different taxonomic levels. The three COS markers used in this study were recommended to us by F. Rodríguez (pers. comm.) and were selected because they amplified easily, produced single-banded PCR products in most accessions, and resulted in clean sequences without the need for cloning. Nomenclature of the COSII markers used in this study is taken from Rodríguez et al. (2009). Molecular data are used to 1) confirm the monophyly of the *Micracantha* clade, determine its sister group, and clarify relationships among the species within the group, 2) examine the geographic distribution of species in a phylogenetic context, 3) study the evolution of morphological features such as habit and fruit morphology, and 4) examine the utility of nuclear COSII markers and the plastid *ndhF-rpl32* and *rpl32-trnL* versus the traditionally used nuclear *waxy* and ITS and the plastid *trnT-F* markers in inferring phylogenetic relationships within the section and more broadly in the spiny solanums.

Materials and Methods

Taxon Sampling—This study sampled 39 taxa from *Solanum* subgenus *Leptostemonum*, including the nine species shown by Stern et al. (2011) to constitute the Micracantha clade. Within this clade, 15 accessions were sampled. Sampling from outside the Micracantha clade was focused on groups that formed a polytomy with the Micracantha clade in previous molecular studies of the subgenus *Leptostemonum* (Levin et al. 2006; Stern et al. 2011). Outgroups used to root the phylogeny were chosen from among more distantly related species within the subgenus. All taxa sampled, 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). The PCR 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). The PCR 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*.

We used the primers for the *rpl32-trnL* and *ndhF-rpl32* markers described by Miller et al. (2009) in PCR reactions of 15 μL each containing 1.5 μL 10 × Mg-free buffer, 1.5 mmol/L MgCl2, 0.25 mmol/L dNTPs, 0.08 umol/L of each primer, 0.7 μL DNA, and 1 unit of AmpliTaq Gold *Taq* polymerase (Applied Biosystems Inc., Foster City, California) and the *waxy* PCR program described in Levin et al. (2005).

Amplification of the three COSII markers was achieved using the following primers: cos4Bf-TTC TTC ATC GCT GCT CAT CTT GC and cos4Br-AGA GGG TTT TTT CTG ACC CAA GAC, cos5Cf- TTG CTT ACT CTT GGT GGA ACA TTC and cos5Cr-TGT CTG TGA TAT CCT CTC TTC TTC, and cos5f-AGC CTA TTT TGA ACT CAA AGA TCT TG and cos5r-TTC TCT CGA CTT TGG CAA TCC ATC. PCR reactions of 15 μL described above along with the *waxy* PCR program described in Levin et al. (2005) were used to amplify the COSII markers.

The PCR 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.

Sequence Alignment and Analysis—Sequence alignments for all of the gene regions were straightforward and performed visually using Se-Al (Rambaut 1996). Because of the disparate size and substitution rates between datasets, the partition homogeneity test (ILD test; Farris et al. 1994; Farris et al. 1995) was not deemed appropriate. Instead, to assess congruence among datasets, each DNA sequence region was analyzed

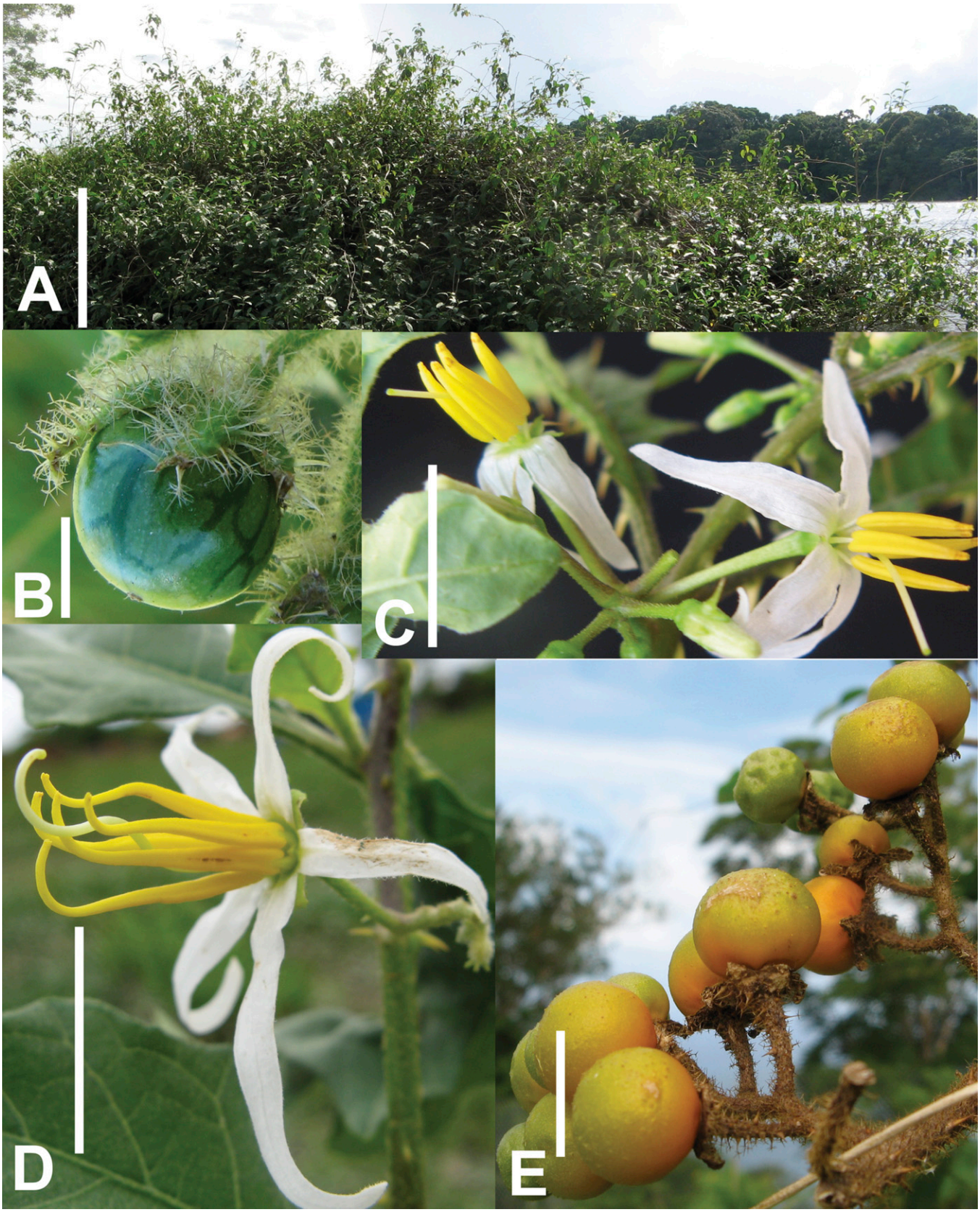

FIG. 2. Morphology of *Solanum* sect. *Micracantha* A. The sprawling, tangled growth form and riverine habit of *S. monachophyllum* (*Stern 256*, UT) on the Potaro River, Guyana. The plant was rhizomatous from a thick woody base and was growing on a sandbar three meters below the high water mark. Scale bar = 1 m. B. Immature fruits of *S. jamaicense* in Trinidad (*Stern 265*, UT). Scale bar = 5 mm. C. Flowers of *S. tampicense* in Florida, U.S.A. (*Bohs & Stern 3655*, UT). Note the style deflected to one side. Scale bar = 5 mm. D. Flowers of *S. arachnidanthum* in Bolivia (*McClelland & Stern 412*, NY). Scale bar = 1.5 cm. E. Infructescence of *S. volubile* in Tobago (*Stern 260*, UT). Scale bar = 1 cm.

individually in both parsimony and Bayesian analyses and the resulting phylogenies were compared to identify regions of conflict using Wiens' (1998) criteria.

Parsimony Analyses—Parsimony analyses were performed on each dataset separately and on the combined dataset using PAUP*4.0b10 (Swofford 2002). All characters were weighted equally in analyses that implemented 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 tree bisection and reconnection (TBR) branch swapping limited to 1,000,000 swaps per replicate.

Bayesian Analyses—Prior to Bayesian analyses, a general model of nucleotide evolution was selected for the separate and the combined datasets using the AIC criterion identified in Modeltest 3.7 (Posada and Crandall 1998). MrBayes 3.1 (Huelsenbeck and Ronquist 2001) was used for Bayesian analysis. For each analysis, five million generations were run using eight Markov chains, each initiated from a random tree and sampled every 1,000 generations. Each of the analyses reached a standard deviation below 0.01 between the chains and all parameters from each analysis were visualized graphically to determine the trees discarded as burn-in prior to achieving stationarity.

Results

Plastid ndhF-rpl32, rpl32-trnL, and trnT-F Datasets—The three plastid regions had the fewest number and lowest percentages of parsimony-informative characters (PIC) of the datasets (Table 1). Despite the low number of PIC, the *trnT-F* dataset strongly resolved more nodes than either of the other plastid markers; these included nodes at the tips as well as in deeper levels of the phylogeny. Importantly, *trnT-F* and *rpl32-trnL* offer strong support for the monophyly of the Micracantha clade, confirming the results of previous studies (e.g. Levin et al. 2006; Stern et al. 2011).

Nuclear ITS and waxy Datasets—The ITS region had the highest percentage of PIC of all the datasets (18.6%). The phylogeny for ITS alone was poorly resolved with few strongly supported nodes in both maximum parsimony (MP) and Bayesian (BI) analyses. The nodes resolved with ITS support species pairs at the tips (except the 1.0 posterior probability [PP] supporting the Micracantha clade in BI). The *waxy* dataset had a moderate level of PIC (9.4%) but had good resolution in both MP and BI. The strongly supported nodes were also spread throughout the topology, from nodes joining species pairs to those joining different sections. The *waxy* phylogeny also supported the monophyly of the Micracantha clade (100% BS, 1.0 PP). Neither of these datasets resolved the sister group to the Micracantha clade. Despite the varying phylogenetic signal strength among the datasets the overall topologies were not conflicting, largely due to lack of resolution and support.

COSII Datasets—The three COSII markers all had high percentages of PIC and resolved moderate to high numbers of strongly supported nodes (Table 1). More importantly, these strongly supported nodes were often at higher taxonomic levels, giving support to relationships between clades that were largely absent in the ITS and chloroplast phylogenies.

The COS4B and COS5C datasets resolved the Micracantha clade as sister to the Bahamense clade. The COS5 dataset placed the Bahamense clade in a polytomy with the Micracantha clade but showed Micracantha + Bahamense to be strongly supported (100% BS, 1.0 PP). Unlike the other markers, the COSII markers had increased resolution and support for the backbone of the phylogeny.

All Datasets—All datasets sequenced well and missing data comprised only 0.012% (40 of 330,026 bases). Analysis of the individual DNA sequence regions consistently identified the same major well-supported clades. Although datasets varied in support and resolution, no strongly supported or "hard" incongruence was found, and thus they are not considered to be conflicting under Wiens' (1998) criteria. The combined MP and BI analyses both strongly support the Micracantha clade (100% BS, 1.0 PP) and place it as sister to the Bahamense clade (100% BS, 1.0 PP; Fig. 3). Both analyses also resolve the Torva, Asterophorum, Old World + Elaeagnifolium, and Crinitum clades as monophyletic (Fig. 3). *Solanum hieronymi* is placed within the Carolinense clade, as in the plastid phylogeny of Wahlert et al. (2014). The relationships among these clades are better resolved than in the phylogenies of Levin et al. (2006) and Stern et al. (2011), with the Torva + Asterophorum clades and Old World + Elaeagnifolium + Carolinense clades resolved as sister groups; these relationships, however, have low BS support. Likewise, *S. campechiense* is resolved as sister to the Micracantha + Bahamense clades, but with modest BS support (74%).

The relationships between species in the Micracantha clade have very strong support in the BI tree (all 1.0 PP) but have lower BS support in the MP trees. The MP analysis of the combined datasets resulted in two most parsimonious trees but these differed in their topologies within the Micracantha clade, causing two nodes to collapse in the MP

Table 1. 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. Names for the COSII markers are from Rodríguez et al. (2009) with the designations from the Sol Genomics Network (SGN; Mueller et al. 2005) given in parenthesis. PIC = Parsimony-Informative Characters, MPT = Most Parsimonious Trees, CI = Consistency Index, RI = Retention Index.

	trnT-F	*rpl32-trnL*	*ndhF-rpl32*	ITS	GBSSI/ *waxy*	COS 4B (At1g44760)	COS 5C (At1g50020)	COS 5 (At2g15890)	All Combined
Aligned Length	1,988	1,244	977	678	1742	1,080	1,450	1,193	10,352
PIC (percent)	81 (4.1)	122 (9.8)	78 (7.9)	126 (18.6)	163 (9.4)	180 (16.7)	208 (14.3)	177 (14.8)	1,135 (11.0)
Parsimony Analysis									
Number of MPT	322,086	351	2,301	45	48	42	81	28,251	2
Length of MPT	286	414	258	605	535	703	675	520	4,263
CI, RI	0.888, 0.879	0.737, 0.748	0.752, 0.748	0.517, 0.578	0.847, 0.870	0.775, 0.779	0.810, 0.842	0.812, 0.882	0.711, 0.734
Number of strongly supported nodes	7	4	6	4	14	7	15	13	20
Bayesian Analysis									
Model	K81uf + G	K81uf + I + G	TIM + I + G	TIM + I + G	HKY + G	TVM + G	GTR + G	TVM + G	GTR + I + G
Number strongly supported nodes	17	21	10	12	24	16	22	17	33

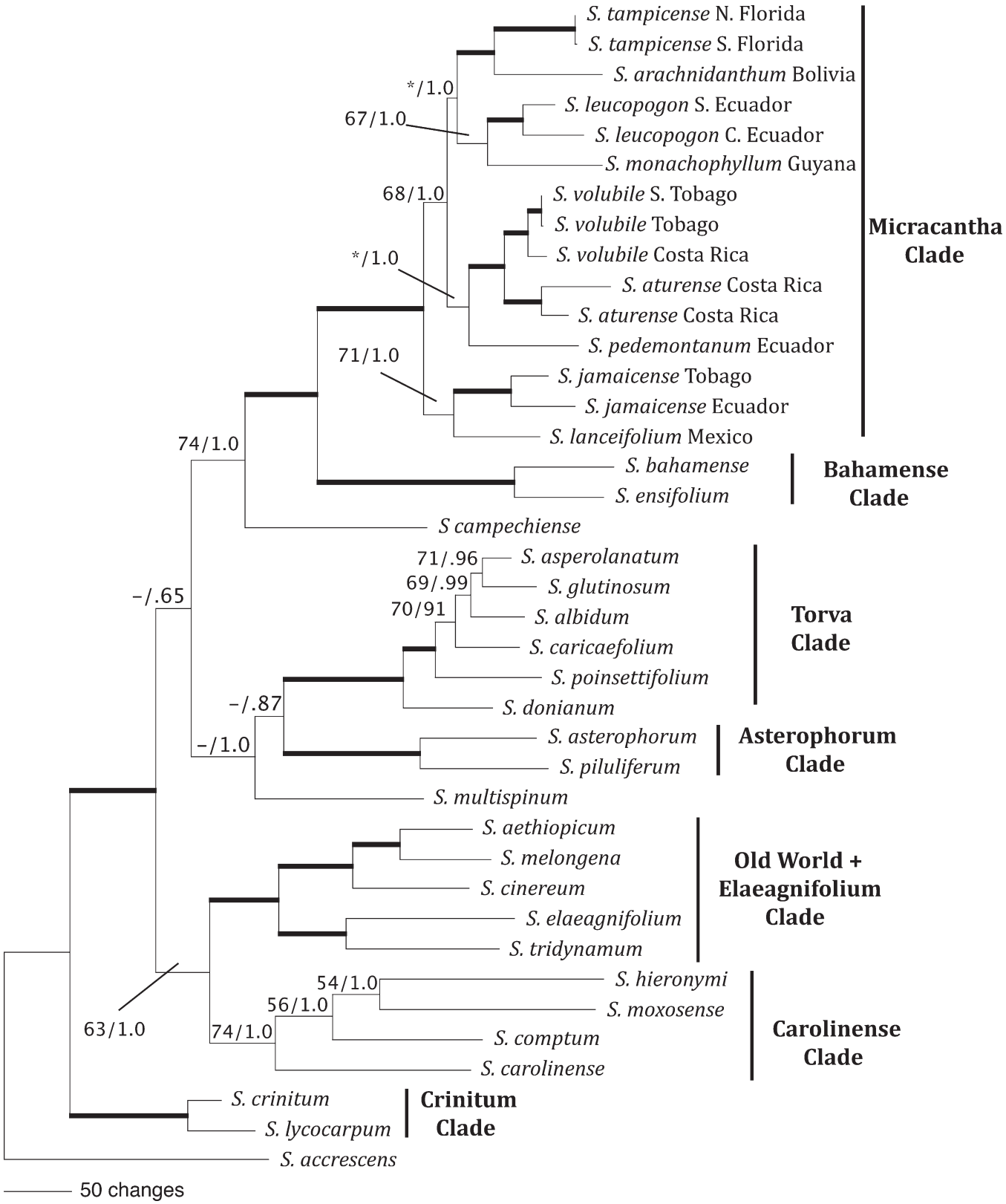

FIG. 3. The 50% majority rule tree from the Bayesian analysis of the combined dataset. Bootstrap and posterior probabilities are shown above the branches. Thickened branches indicate nodes with > 90% BS and > 0.95 PP. Asterisks indicate nodes that collapse in the MP strict consensus tree and dashes indicate BS values below 50%. Clade names are indicated following Levin et al. (2006) and Stern et al. (2011).

strict consensus phylogeny (see asterisks, Fig. 3). Multiple accessions were sampled for five species within the Micracantha clade, (*S. tampicense, S. leucopogon, S. volubile, S. aturense,* and *S. jamaicense*). In each case, the species was resolved as monophyletic. Strongly supported groups within the Micracantha clade in the combined analysis included *S. tampicense* + *S. arachnidanthum* (100% BS, 1.0 PP) and *S. volubile* + *S. aturense* (99% BS, 1.0 PP). BI strongly supported *S. leucopogon* + *S. monachophyllum* (1.0 PP), a clade consisting of these two species plus *S. tampicense* and *S. arachnidanthum* (1.0 PP), *S. jamaicense* + *S. lanceifolium* (1.0 PP), and a clade composed of *S. volubile, S. aturense*, and *S. pedemontanum* (1.0 PP), but bootstrap support for these groups was moderate to low.

Discussion

Monophyly of the Micracantha Clade and its Sister Group in Subgenus Leptostemonum—Multiple individual datasets (COS4B, COS5C, ITS, *waxy*, *rpl32-trnL*, and *trnT-F*) along with the combined MP and BI analyses all support the monophyly of the Micracantha clade. The circumscription of the Micracantha clade in this study includes *S. arachnidanthum, S. aturense, S. jamaicense, S. lanceifolium, S. leucopogon, S. monachophyllum, S. pedemontanum, S. tampicense,* and *S. volubile* (Fig. 2). Morphological characters including recurved prickles, difoliate sympodial units, unbranched inflorescences (except in *S. asperrimum*), deeply stellate corollas with strap-shaped lobes and very little interpetalar tissue, and glabrous fruits with small seeds with a unique anticlinal cell wall arrangement support the inclusion of *S. apaporanum* R. E. Schult., *S. asperrimum* Bitter & Moritz, and *S. flexicaule* Benth. in the group, but these were not sampled with molecular data. Our circumscription is very similar to Whalen's (1984), which formed a subset of Nee's (1999) more inclusive interpretation of the section (Fig. 1). Like Nee (1999), we also include *S. jamaicense* in sect. *Micracantha*, whereas Whalen (1984) left it unplaced. This study and those of Levin et al. (2006) and Stern et al. (2011) confirm its placement in the Micracantha clade.

Previous molecular studies (Levin et al. 2006; Stern et al. 2011) show that *S. coriaceum, S. nemorense, S. paraibanum, S. poinsettiifolium, S. sendtnerianum,* and *S. subinerme*, all of which Nee (1999) included in his sect. *Micracantha*, actually belong in other clades. *Solanum barbeyanum* was not sampled in Levin et al. (2006) or Stern et al. (2011) but was placed in the same species group (*S. nemorense* group; Whalen 1984) and subsection (Nee 1999) as *S. nemorense,* and the two species are obviously closely related. They both lack the stellate hairs typical of subgenus *Leptostemonum* and share broad-based recurved prickles on the stems, petioles, inflorescence axes, and leaf midveins, often prickly leaf margins, and large orange fruits. Both Levin et al. (2006) and Stern et al. (2011) show that *S. nemorense* is far removed from the Micracantha clade, and it follows that *S. barbeyanum* can also safely be excluded from it on morphological grounds.

Based on morphological characteristics, including pubescent fruits and unifoliate sympodia, it appears that *S. nuricum*, another species that Nee (1999) included in his circumscription of sect. *Micracantha*, belongs in the Thomasiifolium clade along with *S. paraibanum* (Stern et al. 2011). In the description of *S. nuricum*, Nee (1994) indicated it was most morphologically similar to *S. rupincola*, a species also placed in the Thomasiifolium clade in Stern et al. (2011).

In this analysis, the sister group to the Micracantha clade is the Bahamense clade, a group of three Caribbean taxa. Our sampling contains two of the species in this group, *S. bahamense* and *S. ensifolium*. While this relationship is strongly supported, it should be noted that there remain many taxa endemic to the Caribbean that have not been sampled in molecular studies. It is possible that the *S. bahamense* group is more closely related to these taxa than it is to the Micracantha clade (Knapp 2009; Strickland-Constable et al. 2010). Species in the Bahamense clade are shrubs with usually unbranched inflorescences, stellate corollas, and glabrous fruits similar to members of the Micracantha clade, but they differ in their strongly recurved fruiting pedicels and the presence of stellate hairs on the adaxial anther surfaces (Whalen 1984; Strickland-Constable et al. 2010).

Within the Micracantha clade, *S. jamaicense* + *S. lanceifolium* are sister to the remainder of species in the group. Both of these species are found in Mexico and Central and South America, but also range widely throughout the Caribbean. The relationship between the Micracantha clade and the Caribbean Bahamense clade may suggest a Caribbean origin for the Micracantha clade, with subsequent expansions into Central and South America. Alternatively, the clade could have arisen in Central or South America with radiations into the Caribbean. Further sampling of the Caribbean endemic spiny solanums may shed light on this question.

Relationships within the Micracantha Clade and Geographic Distribution of Species in a Phylogenetic Context—The species pair of *S. aturense* + *S. volubile* is strongly supported in the combined phylogeny and both are common throughout Central America and into northwestern South America. The southern limits of their distributions occur near the border of Ecuador and Colombia, which is the northern limit of their sister species, *S. pedemontanum*. These three species are the most robust members of the section and are vines of disturbed habitats that can reach 20 m in length. They also share similar large, leathery fruits. *Solanum leucopogon* also shares these morphological characters and has a similar geographical distribution to *S. pedemontanum* in the eastern Andean foothills of Ecuador, Peru, and Bolivia. Surprisingly, however, *S. leucopogon* is sister to *S. monachophyllum*, but with low bootstrap support. *Solanum monachophyllum* is a species of riverine habitats in the white sands of the Guiana Shield and Venezeula. Morphologically this is also an unusual relationship because *S. leucopogon* can grow to be a vine 10 m in length while *S. monachophyllum* is a small rhizomatous shrub.

Solanum arachnidanthum has the most southerly distribution of the species in the clade. It is sister to *S. tampicense*, which is found in the Caribbean and Central America and reaches the northernmost distribution of the clade in southern Florida. From a morphological standpoint this relationship is understandable as both have small, thin-skinned berries, a scandent growth form, and grow in swampy, seasonally flooded areas. It is possible that their large geographical disjunction is a result of long distance bird dispersal of the small, red berries. Disjunct distributions are not without precedent in *Solanum*. For example, species of the Carolinense clade occur in North and South America (Wahlert et al. 2014) and *Solanum elaeagnifolium* is found in both southern South America and the southwestern USA and Mexico (Levin et al. 2006).

Morphology of sect. Micracantha and the Evolution of Habit and Fruit Morphology—All species of *Solanum* sect.

Micracantha share the following combination of characters: 1) they are scandent shrubs or woody vines with recurved prickles; 2) mature, flowering portions of the stem have difoliate sympodial units; 3) the inflorescences are unbranched (except in *S. asperrimum*); 4) the corollas are deeply stellate with strap-shaped lobes and very little interpetalar tissue; 5) the fruits are orange to red, glabrous, and contain small seeds with a unique anticlinal cell wall arrangement where each cell has numerous invaginations giving an "amoeboid" appearance. Although other clades have one or more of these characteristics, this combination of characters is unique in the genus. While some characters vary within the clade, such as habit and fruit characteristics described below, other characteristics, like fruit color or pubescence, are constant within sect. *Micracantha*.

The habit of species in the Micracantha clade appears not to be strongly correlated with phylogeny but instead may be more tied to the habitat in which species grow, with those from swampy areas having a sprawling or scandent growth form while those of forest edges or gaps tending to be climbing vines. Variation in habit can be difficult to assess from herbarium collections due to lack of label data. Field study has shown that three taxa in the clade, *S. tampicense, S. arachnidanthum,* and *S. monachophyllum,* are species of riverine or swampy habitats and have a sprawling growth form, similar to the brambling, festooning growth form of the genus *Rubus* L. *Solanum jamaicense* is the only species that is consistently a free-standing shrub. *Solanum aturense* and *S. pedemontanum* can vary from self-supporting to opportunistically vining plants. The more obligate vining species, including *S. apaporanum, S. lanceifolium*, *S. leucopogon* and *S. volubile*, may be self-supported as young plants but quickly climb up other plants. While a vining habit has been important in circumscribing sect. *Micracantha*, Stern et al. (2011) showed that a vining habit has also evolved multiple times in various clades of *Solanum* subgenus *Leptostemonum* (Stern et al. 2011).

The fruits of species in the Micracantha clade are all fleshy berries but range from small and thin-walled (e.g. *S. arachnidanthum, S. jamaicense, S. lanceifolium, S. monachophyllum*, *S. tampicense*) to larger and leathery in texture (*S. aturense, S. leucopogon, S. pedemontanum,* and *S. volubile*). Members of the Bahamense clade have small, thin-walled fruits, and this is likely the ancestral state in the Micracantha clade. According to the phylogeny, large leathery fruits have evolved at least twice within the Micracantha clade. Fruit dispersal has not been well studied in *Solanum*, particularly in sect. *Micracantha*. The small, colorful berries suggest bird dispersal. It is possible that larger, leathery fruits may have evolved for mammal, particularly bat, dispersal but this remains to be observed in the field.

Utility of COSII Markers, rpl32-trnL, and ndhF-rpl32— While the *trnT-F,* ITS, and *waxy* markers have been used in multiple studies in the genus *Solanum*, COSII markers and the chloroplast *rpl32-trnL* and *ndhF-rpl32* regions are relatively new for phylogenetic study in the genus. The COSII markers proved to be a valuable tool for improving phylogenetic resolution. They have higher percentages of PIC than the chloroplast markers and *waxy* (Table 1). The COSII markers amplify easily, have single pass sequencing, and provided strong phylogenetic resolution in the present study, making them valuable tools for phylogenetic study, as has been found in other studies of the Solanaceae (Levin et al. 2009; Rodríguez et al. 2009). In contrast, Tepe and Bohs (2010) found that when COSII markers were used individually they gave poor resolution, poor support, or conflicting topologies. Because COSII markers are still in development, the findings of Tepe and Bohs (2010) suggest that careful screening and attention to topological signal is important when using COSII markers for phylogenetic analysis.

Addition of the COSII and chloroplast markers to the *waxy, trnT-F*, and ITS data that have traditionally been used in phylogenetic research in the genus *Solanum* increased the resolution and support for the relationships among major clades in subgenus *Leptostemonum.* In particular, the new markers strongly supported the Bahamense clade as the sister group to the Micracantha clade and substantially increased resolution of the tree backbone compared to the previous studies of Levin et al. (2006) and Stern et al. (2011). Future studies using the expanded set of markers as well as additional taxon sampling are expected to further clarify phylogenetic relationships within the spiny solanums.

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Appendix 1. List of taxa and voucher specimens used in this study, in the order: taxon, provenance, collector, collection number, (herbarium where the voucher is deposited); GenBank accession numbers given in the order: ITS, waxy, trnT-F, *trnL-rpl32*, *rpl32-ndhF*, COS4b, COS5c, COS5. BIRM and Nijmegen samples have the seed accession number for the Solanaceae collections at the University of Birmingham, U. K., and Radboud University, Nijmegen, The Netherlands, respectively.

Solanum accrescens Standl. & C.V. Morton, Costa Rica, *Bohs 2556* (UT); AY996480, AY996375, DQ180473, HQ457200, HQ457239, HQ457278, HQ457317, HQ457356. ***Solanum aethiopicum*** L., BIRM S.0344, *Olmstead S-74* (WTU); AY996482, AY996378, DQ180394, HQ457202, HQ457240, HQ457279, HQ457318, HQ457357. ***Solanum albidum*** Dunal, Bolivia, *Nee 51831* (NY); GU591056, GU591108, GU590997, HQ457202, HQ457241, HQ457280, HQ457319, HQ457358. ***Solanum arachnidanthum*** Rusby, Bolivia, *McClelland & Stern 412* (NY); GU591057, GU591109, GU590998, HQ457203, HQ457242, HQ457281, HQ457320, HQ457359. ***Solanum asperolanatum*** Ruiz & Pav., Bolivia, *Nee 51761* (UT); GU591058, GU591110, GU590999, HQ457204, HQ457243, HQ457282, HQ457321, HQ457360. ***Solanum asterophorum*** Mart., Brazil, *Agra et al. 7210* (JPB); GU591059, GU591111, GU591000, HQ457205, HQ457244, HQ457283, HQ457322, HQ457361. ***Solanum aturense*** Dunal, Costa Rica, *Soto et al. 1219* (UT); GU799062, GU799072, GU799082, HQ457206, HQ457245, HQ457284, HQ457323, HQ457362. Costa Rica, *Bohs 2976* (UT); AY996487, AY996386, GQ149730, HQ457207, HQ457246, HQ457285, HQ457324, HQ457363. ***Solanum bahamense*** L., Nijmegen 944750187, *Bohs 2936* (UT); AY996487, AY996386, GQ149730, HQ457208, HQ457247, HQ457286, HQ457325, HQ457364. ***Solanum campechiense*** L., Costa Rica, *Bohs 2536* (UT); AF244728, AY996389, DQ180475, HQ457209, HQ457248, HQ457287, HQ457326, HQ457365. ***Solanum caricaefolium*** Rusby, Bolivia, *Bohs 2741* (UT); GU591064, GU591116, GU591006, HQ457210, HQ457249, HQ457288, HQ457327, HQ457366. ***Solanum carolinense*** L., BIRM S.1816, *Olmstead S-77* (WTU); AY996491, AY996392, DQ180476, HQ457211, HQ457250, HQ457289, HQ457328, HQ457367. ***Solanum cinereum*** R. Br., Nijmegen 904750120, *Bohs 2852* (UT); AY996493, AY996394, DQ180397, HQ457212, HQ457251, HQ457290, HQ457329, HQ457368. ***Solanum comptum*** C.V. Morton, Paraguay, *Bohs 3193* (UT); AY996498, AY996399, GU591009, HQ457213, HQ457252, HQ457291, HQ457330, HQ457369. ***Solanum crinitum*** Lam., Brazil, *Agra et al. 7028* (JPB); GQ143651, GQ143683, GQ149736, HQ457214, HQ457253, HQ457292, HQ457331, HQ457370. ***Solanum donianum*** Walp., Mexico, *Bohs 3472* (UT); GU591069, GU591121, GU591013, HQ457215, HQ457254, HQ457293, HQ457332, HQ457371. ***Solanum elaeagnifolium*** Cav., Paraguay, *Bohs 3204* (UT); AY996508, AY996412, DQ180399, HQ457216, HQ457255, HQ457294, HQ457333, HQ457372. ***Solanum ensifolium*** Dunal, Puerto Rico, *Bohs 2461* (UT); AY996506, AY996409, DQ180483, HQ457217, HQ457256, HQ457295, HQ457334, HQ457373. ***Solanum glutinosum*** Dunal, Nijmegen A34750191, *Bohs 3262* (UT); AY996513, AY996419, GU591016, HQ457218, HQ457257, HQ457296, HQ457335, HQ457374. ***Solanum hieronymi*** Kuntze, Argentina, *Nee & Bohs 50761* (NY); AY996517, AY996423, GU591019, HQ457219, HQ457258, HQ457297, HQ457336, HQ457375. ***Solanum jamaicense*** Mill., Trinidad, *Stern 265* (UT); GU799063, GU799073, GU799083, HQ457220, HQ457259, HQ457298, HQ457337, HQ457376. Ecuador, *Stern & Tepe 389* (UT); GU799064, GU799074, GU799084, HQ457221, HQ457260, HQ457299, HQ457338, HQ457377. ***Solanum lanceifolium*** Jacq., Mexico, *Aguilar et al. 1130* (MO); GU591075, GU591127, GU591022, HQ457222, HQ457261, HQ457300, HQ457339, HQ457378. ***Solanum leucopogon*** Huber, Ecuador (southern), *Bohs et al. 3364* (UT); GU799065, GU799075, GU799085, HQ457223, HQ457262, HQ457301, HQ457340, HQ457379. Ecuador (central), *Stern & Tepe 271* (UT); GU799068, GU799078, GU799088, HQ457224, HQ457263, HQ457302, HQ457341, HQ457380. ***Solanum lycocarpum*** A. St.-Hil., Paraguay *Bohs 3212* (UT); AY996525, AY996435, DQ812107, HQ457225, HQ457264, HQ457303, HQ457342, HQ457381. ***Solanum melongena*** L., BIRM S.0657, *Olmstead S-91* (WTU); GU591078, AY562959, DQ180406, HQ457226, HQ457265, HQ457304, HQ457343, HQ457382. ***Solanum monachophyllum*** Dunal, Guyana, *Stern 256* (UT); GU591079, GU591130, GU591027, HQ457227, HQ457266, HQ457305, HQ457344, HQ457383. ***Solanum moxosense*** M. Nee, Bolivia, *McClelland & Stern 414* (NY); GU591081, GU591132, GU591029, HQ457228, HQ457267, HQ457306, HQ457345, HQ457384. ***Solanum multispinum*** N.E. Br., Paraguay, *Bohs 3198* (UT); AY996533, AY996444, GU591030, HQ457229, HQ457268, HQ457307, HQ457346, HQ457385. ***Solanum pedemontanum*** M. Nee, Ecuador, *Bohs 3337* (UT); GU591084, GU591135, GU591034, HQ457230, HQ457269, HQ457308, HQ457347, HQ457386. ***Solanum piluliferum*** Dunal, Brazil, *Agra 7280* (JPB); HQ457398, HQ457417, HQ457407, HQ457231, HQ457270, HQ457309, HQ457348, HQ457387. ***Solanum poinsettifolium*** Rusby, Bolivia, *McClelland & Stern 414* (NY); GU591086, GU591137, GU591036, HQ457232, HQ457271, HQ457310, HQ457349, HQ457388. ***Solanum tampicense*** Dunal, Florida (south), *Bohs & Stern 3655* (UT); GU799069, GU799079, GU799089, HQ457233, HQ457272, HQ457311, HQ457350, HQ457389. Florida (north), no voucher; GU591097, GU591148, GU591047, HQ457234, HQ457273, HQ457312, HQ457351, HQ457390. ***Solanum tridynamum*** Dunal, Nijmegen 904750179, *Bohs 2977* (UT); GU591101, AY996474, DQ180412, HQ457235, HQ457274, HQ457313, HQ457352, HQ457391. ***Solanum volubile*** Sw., Tobago (south), *Stern 263*; GU799060, GU799070, GU799080, HQ457236, HQ457275, HQ457314, HQ457353, HQ457392. Tobago (north), *Stern 260* (UT); GU799061, GU799071, GU799081, HQ457237, HQ457276, HQ457315, HQ457354, HQ457393. Costa Rica, *Bohs 2473* (UT); AF244723, AY996377, DQ180474, HQ457238, HQ457277, HQ457316, HQ457355, HQ457394.