Molecular delimitation of clades within New World species of the "spiny solanums" (*Solanum* subg. *Leptostemonum*)

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Abstract *Solanum* subg. *Leptostemonum* contains approximately 350–450 species, including the cultivated eggplant, *S. melongena*. Most species placed in this subgenus form a monophyletic group, the Leptostemonum clade, characterized by the presence of stellate hairs and prickles, leading to the common name of "spiny solanums". Here we present a phylogenetic analysis that circumscribes the major clades within the spiny solanums and examines the relationships among them, with an emphasis on New World species. Of particular interest is the clarification of the clade limits and species composition of groups that have not been well-sampled. We also increase sampling of taxa that have been previously analyzed in molecular studies, namely those in the Torva, Micracantha, and Erythrotrichum clades. These groups have convergent morphological characteristics that have challenged taxonomists, making classification difficult. Results from our study delimit 14 clades within the spiny solanums, including the newly designated Asterophorum, Gardneri, Sisymbriifolium, and Thomasiifolium clades. We also establish the placement of species not previously sampled, especially those endemic to Brazil. These results give an increased understanding of the evolution of the Leptostemonum clade by defining monophyletic groups within it and identify areas of the phylogenetic tree that remain unresolved and require further taxon sampling.

Keywords granule-bound starch synthase (GBSSI); ITS; *Leptostemonum*; Solanaceae; *Solanum*; *trnT-F*; *waxy*

Supplementary Material Figures S1–S3 are available in the free Electronic Supplement to the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

INTRODUCTION

Solanum L. (Solanaceae), with approximately 1400 species, is one of the 10 largest genera of flowering plants and contains economically important species such as the tomato (*S. lycopersicum* L.), eggplant (*S. melongena* L.), and potato (*S. tuberosum* L.) (Frodin, 2004; Bohs, 2005). Recent studies have aimed to resolve phylogenetic relationships within *Solanum* as well as clarify species-level taxonomy (Knapp & al., 2004; Bohs, 2005; Weese & Bohs, 2007; www.solanaceaesource.org). Analyses of DNA sequence data have helped to identify the major monophyletic groups within *Solanum*, the largest of which is the Leptostemonum clade with approximately 350–450 species (Bohs, 2005; Levin & al., 2006). This clade is characterized by the presence of stellate hairs and prickles, leading to the common name of "spiny solanums". The Leptostemonum clade largely conforms to the traditionally recognized *Solanum* subg. *Leptostemonum* (Dunal) Bitter with the exclusion of the *S. wendlandii* and *S. nemorense* species groups, whose members lack stellate hairs (Whalen, 1984; Levin & al., 2006). Hereafter, we will refer to this group as the Leptostemonum clade.

The species of the Leptostemonum clade have a worldwide distribution. Whalen (1984) notes three centers of diversity, including the Neotropics (ca. 250 spp.), Africa (ca. 80 spp.), and Australia (ca. 150 spp.). Previous authors have used morphological characters to define groups within the spiny solanums. D'Arcy (1972) recognized 22 sections; however, because he did not explicitly state species composition of these groups, comparison with other classifications is difficult. Whalen's (1984) treatment informally recognized 33 species groups with 36 species left unplaced. Nee (1999) treated only the New World species of spiny solanums, which are the major focus of this study, and placed them into 10 sections. Because we are primarily concerned with delimiting clades, elucidating their species composition, and placing previously unsampled taxa in a phylogenetic tree, we will refer to Whalen's (1984) and Nee's (1999) classifications since they both explicitly specify which species belong in each of their groups.

Levin & al. (2006) sampled species of the Leptostemonum clade from throughout its distribution for three molecular markers, the chloroplast *trnS-G* and the nuclear ITS and GBSSI or *waxy*, to construct a molecular phylogeny of the spiny solanums. This study delimited the Leptostemonum clade and defined 10 major clades within it. One of these clades is composed exclusively of taxa from the Old World. The Old World clade is the topic of other studies (Martine & al., 2006; L. Bohs & al., unpub. data) and only a few representatives are included here as placeholders. Within the New World, nine well-supported clades were designated, but the relationships among them were not well resolved (Levin & al., 2006). In addition to its utility as an overview, Levin & al. (2006) also included more exhaustive data for specific clades, such as the Lasiocarpa and Acanthophora clades (Bohs, 2004; Levin & al., 2005).

A goal of the current study is to increase taxon sampling for many of the large groups underrepresented in Levin & al. (2006) with an emphasis on New World species. Our phylogenetic analysis targets species from sections that have not previously been studied, such as sect. *Polytrichum* (Whalen) Child, and samples more intensively from three clades that were previously underrepresented in molecular studies (the Torva, Micracantha, and Erythrotrichum clades). These groups have unclear limits and species compositions that have varied in previous classifications. In Nee's (1999) classification, these three clades account for 82 of the 185 species (44%) of the New World spiny solanums. Additionally, our phylogenetic analysis samples from geographic areas that have been underrepresented, including Brazil and the Caribbean, and specifically targets species in sect. *Polytrichum* and sect. *Persicariae* Dunal that are endemic to these areas.

In this paper, we also aim to increase resolution and support of clades within the spiny solanums by using a longer and more variable chloroplast marker, *trnT-F*, in place of *trnS-G* used in Levin & al.'s (2006) study. Comparisons of large scale phylogenies within the genus *Solanum* show that *trnT-F* is nearly twice the length and contains almost twice the number of parsimony-informative characters than *trnS-G* (Levin & al., 2005, 2006; Weese & Bohs, 2007). We compare our phylogenetic results with previous morphological classifications, and, where possible, identify morphological characters associated with each clade.

■ MATERIALS AND METHODS

Taxon sampling. — This study sampled 102 taxa from the Leptostemonum clade. These represent 44 of the 112 species (39%) of the clade sampled in Levin & al. (2006). We sampled 58 additional species of spiny solanums from groups that were not well represented in Levin & al. (2006). Selected species from clades that Levin & al. (2006) sampled extensively or that are the focus of other studies were included only to show their higher-level relationships. These groups include the Lasiocarpa clade (Bohs, 2004), the Acanthophora clade (Levin & al., 2005), the Androceras clade (Stern & al., 2010), and the Old World clade (Martine & al., 2006; L. Bohs & al., unpub. data). Focal groups of this study include the Torva, Micracantha, and Erythrotrichum clades. The spiny species *S. polygamum* Vahl was placed within the Leptostemonum clade in Levin & al. (2006) but was not resolved within any of the major clades. It was excluded from this study because its sequences were divergent for all sampled markers, making unambiguous alignment difficult. Spiny solanums in the *S. wendlandii* and *S. nemorense* species groups that have been shown to lie outside of the Leptostemonum clade (Levin & al., 2006) were also included. Non-spiny *Solanum* species in the Geminata (*S. argentinum*, *S. pseudocapsicum*, *S. arboreum*), Brevantherum (*S. abutiloides* and *S. cordovense*), Cyphomandra (*S. betaceum*, *S. diploconos*, *S. glaucophyllum*), Morelloid (*S. ptychanthum*), and Dulcamaroid (*S. dulcamara*) clades sensu Bohs (2005) and Weese & Bohs (2007) were included as outgroups and the tree was rooted using *S. laciniatum*, a member of the Archaesolanum clade, which was previously shown to be even more distantly related to the spiny solanums (Levin & al., 2006; Weese & Bohs, 2007). All taxa, along with voucher information and GenBank accession numbers, are listed in the Appendix.

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, Valencia, California, U.S.A.). PCR amplification for each gene region followed standard procedures described in Taberlet & al. (1991), Bohs & Olmstead (2001), and Bohs (2004) for the *trnT-L* and *trnL-F* intergenic spacer regions; Levin & al. (2005) for *waxy*; and Levin & al. (2006) for ITS. The ITS region was amplified as a single fragment using primers ITSleu1 (Bohs & Olmstead, 2001) and ITS4 (White & al., 1990) using PCR conditions described in Bohs & Olmstead (2001). When possible, *trnT-F* and *waxy* were amplified as single fragments using primers a and f for *trnT-F* (Taberlet & al., 1991) and primers waxyF and waxy2R for *waxy* (Levin & al., 2005). PCR conditions for *trnT-F* followed Bohs & Olmstead (2001); conditions for *waxy* followed Levin & 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*.

PCR products were cleaned using the Promega Wizard SV PCR Clean-Up System (Promega Corporation, Madison, Wisconsin, U.S.A.). The University of Utah DNA Sequencing Core Facility performed sequencing on an ABI automated sequencer. Sequences were edited in Sequencher v.4.8 (Gene Codes Corp., Ann Arbor, Michigan, U.S.A.) and all new sequences were submitted to GenBank (Appendix).

Sequence alignment and analyses. — Sequence alignments for all of the gene regions were straightforward and performed visually using Se-Al v.2.0a11 (Rambaut, 1996). The aligned datasets and representative phylogenetic trees are available in TreeBASE (S11233). Missing data comprised 0.00062% of the combined data matrix (286 of 462,031 total bases).

Parsimony analyses were performed on each dataset separately and on the combined dataset using PAUP* v.4.0b10 (Swofford, 2002). All characters were weighted equally in analyses that implemented tree bisection and reconnection (TBR) branch swapping with 1000 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 1000 random-addition replicates and TBR branch swapping limited to 1,000,000 swaps per replicate. Datasets were further analyzed using TNT v.1.0 (Goloboff & al., 2008) to search for shorter trees than were obtained in standard PAUP* analyses. 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.

Prior to Bayesian analyses, a general model of nucleotide evolution was selected for both the separate and the combined datasets using the Akaike information criterion identified in Modeltest v.3.7 (Posada & Crandall, 1998). MrBayes v.3.1 (Huelsenbeck & Ronquist, 2001) was used to analyze each of the separate and combined datasets. For each analysis, five million generations were run using eight Markov chains, each initiated from a random tree and sampled every 1000 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. The Bayesian analysis reached stationarity and the first 500,000 generations were eliminated as burn-in in all analyses.

■ RESULTS

Phylogenetic analyses. — The parsimony strict consensus and Bayesian majority-rule consensus trees of all datasets differed only in the degree of resolution, with Bayesian tree topologies more resolved than parsimony trees (Table 1). Clades with low posterior probabilities (PP), typically those below 0.90 PP but occasionally those with up to 1.0 PP in Bayesian analyses were often collapsed in parsimony strict consensus trees. Descriptive statistics for individual and combined genes are provided (Table 1). More nodes were strongly supported by combining the data than were obtained in any of the separate analyses.

Topological conflicts. — In parsimony analyses, each DNA sequence region consistently identified the same major, well-supported clades comprising identical species groups, but relationships among major clades were often not strongly supported (BS < 90%), or were unresolved, and thus cannot be considered conflicting under Wiens' (1998) criteria. The Bayesian analyses of individual datasets gave some conflicting nodes (cutoff at <0.95 PP). However, posterior probabilities are known to be inflated relative to bootstrap values (Cummings & al., 2003; Erixon & al., 2003; Simmons & al., 2004). Our discussion will be based on the parsimony strict consensus tree of the combined dataset, which is a conservative hypothesis of phylogenetic relationships (Fig. 1). The parsimony strict consensus trees for the individual markers (Figs. S1–S3) are presented in the Electronic Supplement.

Higher level phylogenetic relationships. — The Leptostemonum clade emerges as monophyletic and strongly supported (100% BS, 1.0 PP). Its overall topology is similar to that of Levin & al. (2006) but our increased taxon sampling and the use of the more informative *trnT-F* marker has given a more resolved backbone and more strongly supported nodes. Our results support the exclusion of the *S. wendlandii* and *S. nemorense* species groups from the Leptostemonum clade as proposed by Levin & al. (2006). These taxa were traditionally placed in *Solanum* subg. *Leptostemonum* because they have prickles. However, the absence of stellate hairs as well as molecular data support their exclusion from the Leptostemonum clade.

With respect to the relationships among the major groups in the Leptostemonum clade, phylogenetic results place the Gardneri clade as sister to the Erythrotrichum+Thomasiifolium clades (99% BS, 1.0 PP). This clade in turn is sister to the remainder of the sampled members of the Leptostemonum clade, which form a monophyletic group with strong support (94% BS, 1.0 PP). Within this latter clade, the Acanthophora and Lasiocarpa clades are sister to each other (85% BS, 1.0 PP). The Androceras/Crinitum clade is weakly supported (63% BS, 1.0 PP) and is weakly supported as sister to the Sisymbriifolium clade (61% BS, 1.0 PP). The Old World clade is weakly supported as sister to the Elaeagnifolium clade (62% BS, 1.0 PP). These two clades plus the New World species *S. hieronymi* form a strongly supported monophyletic group (99% BS, 1.0 PP). The relationships among the Torva, Micracantha, Carolinense, Asterophorum, and Bahamense clades remain unclear. These groups, along with three species unassigned to the 14 major clades, *S. campechiense*, *S. crotonoides*, and *S. multispinum*, form a large but unsupported polytomy.

Sectional level relationships. — The Leptostemonum clade is comprised of 14 large, well-supported clades (63%–100% BS, all 1.0 PP), which largely correspond to those of Levin & al. (2006). There are four taxa that remain unassigned to the 14 clades, *S. campechiense*, *S. crotonoides*, *S. hieronymi*, and *S. multispinum* (arrows, Fig. 1). Table 2 provides a summary of the recognized clades with their bootstrap and posterior probability support values and compares the clade species composition with the species groups of Whalen (1984) and sections of Nee (1999). Each clade is discussed in more detail below.

Table 1. Descriptive statistics for each dataset 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	Parsimony informative characters	Most parsimonious trees	Tree length	Consistency index	Retention index	Strongly supported nodes, parsimony	Model selected	Strongly supported nodes, Bayesian
ITS	711	245	209	1747	0.361	0.660	21	TIM+I+G	54
waxy	2231	505	19,579	1606	0.691	0.838	46	TVM+G	79
trnT-F	2137	224	385,577	706	0.817	0.875	27	GTR+I+G	63
Combined	5079	974	209	4223	0.549	0.742	61	GTR+I+G	82

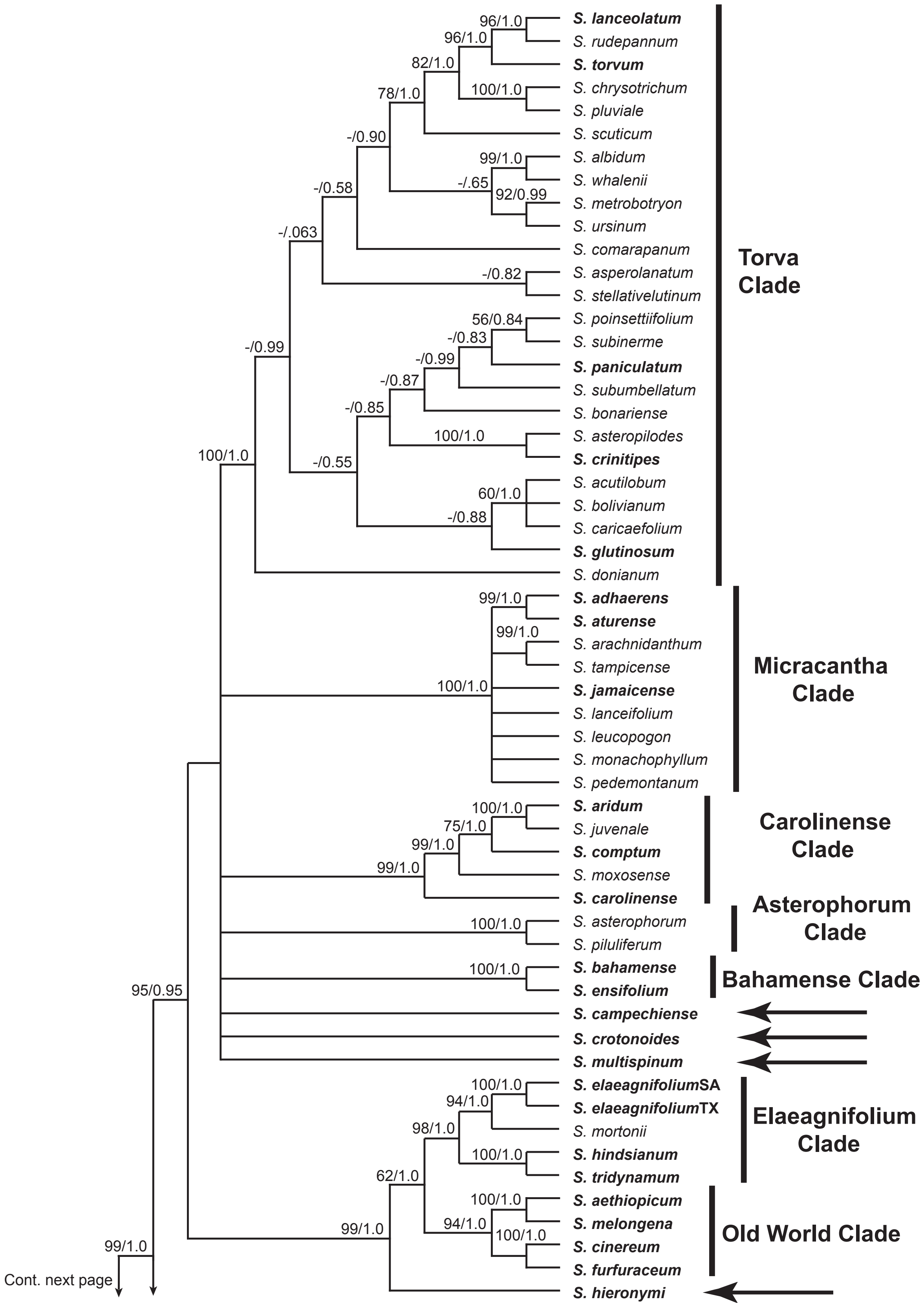
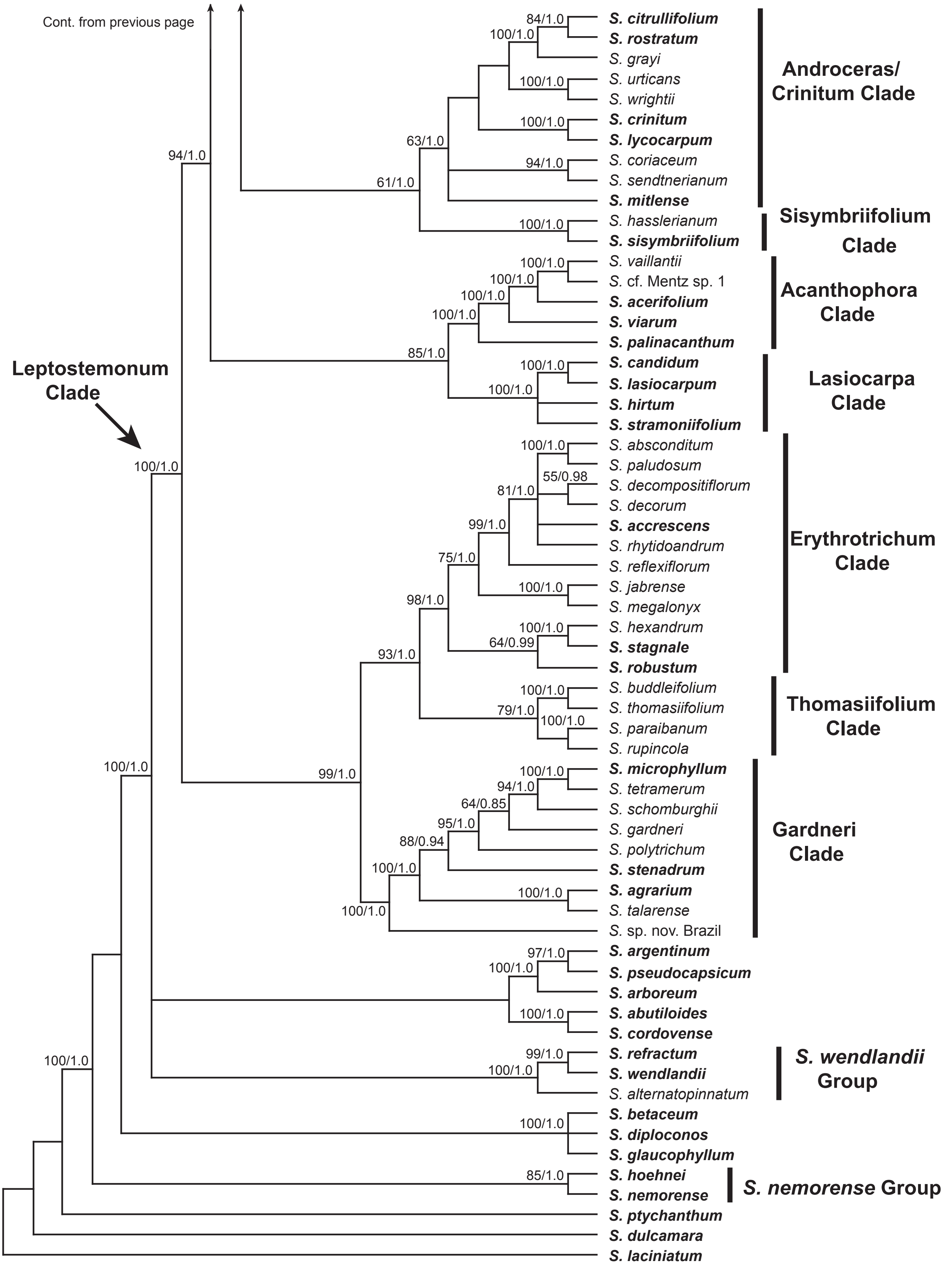


Fig. 1. Strict consensus of 209 most parsimonious trees obtained from the combined analysis of the *trnT-F*, *waxy*, and ITS data. Numbers above branches are bootstrap values over 50% followed by posterior probabilities from Bayesian analysis. Species names in bold italics are those sampled in Levin & al. (2006); species in italics are those new to this study. The clades discussed in the text are labeled. The four taxa that are ▶



▶ unplaced within the 14 clades are indicated by arrows. Taxa in the spiny *S. nemorense* and *S. wendlandii* species groups that are not members of the Leptostemonum clade are labeled, whereas non-spiny outgroups are not labeled. The abbreviations SA and TX for *S. elaeagnifolium* refer to specimens from South America and Texas, respectively.

Table 2. Summary of the 14 clades recognized here with support values. The species composition of each clade is compared with Whalen's (1984) species groups and Nee's (1999) sections. Asterisks indicate instances where representatives from a species group or section are included in more than one clade or emerge in multiple places on the tree. See Discussion for further details.

Clade name (bootstrap support, posterior probability)	Species groups of Whalen (1984) included	Sections of Nee (1999) included
Torva (100% BS, 1.0 PP)	*S. torvum* species group, *S. subinerme* species group, 1 species not treated	Sect. *Torva*, Sect. *Micracantha**
Micracantha (100% BS, 1.0 PP)	*S. lanceifolium* species group, 1 unplaced species	Sect. *Micracantha**
Carolinense (99% BS, 1.0 PP)	*S. multispinum* species group*, 1 unplaced species	Sect. *Melongena**
Asterophorum (100% BS, 1.0 PP)	*S. asterophorum* species group	Sect. *Polytrichum**, 1 species not treated
Bahamense (100% BS, 1.0 PP)	*S. bahamense* species group	Sect. *Persicariae**
Elaeagnifolium (98% BS, 1.0 PP)	*S. ellipticum* species group*, *S. vespertilio* species group*, 1 species not treated, 1 species unplaced	Sect. *Melongena**
Old World (94% BS, 1.0 PP)	Various species groups	Mainly sect. *Melongena**
Androceras/Crinitum (63% BS, 1.0 PP)	*S. rostratum* species group, *S. crinitum* species group, 1 unplaced species, 2 species not treated	Sect. *Androceras*, Sect. *Crinitum*, Sect. *Micracantha**
Sisymbriifolium (100% BS, 1.0 PP)	*S. polytrichum* species group*, 1 unplaced species	Sect. *Melongena**
Acanthophora (100% BS, 1.0 PP)	*S. mammosum* species group*	Sect. *Acanthophora**
Lasiocarpa (100% BS, 1.0 PP)	*S. quitoense* species group	Sect. *Lasiocarpa*
Erythrotrichum (98% BS, 1.0 PP)	*S. erythrotrichum* species group, *S. polytrichum* species group*, 5 species not treated	Sect. *Erythrotrichum**, 6 species not treated
Thomasiifolium (79% BS, 1.0 PP)	*S. polytrichum* species group*, 2 unplaced species, 1 species not treated	Sect. *Persicariae**, Sect. *Erythrotrichum**, Sect. *Micracantha**, 1 species not treated
Gardneri (100% BS, 1.0 PP)	*S. polytrichum* species group*, *S. mammosum* species group*, 3 unplaced species, 3 species not treated	Sect. *Persicariae**, Sect. *Polytrichum**, Sect. *Acanthophora**, 1 species not treated

■ DISCUSSION

Our increased taxon sampling and the use of the more phylogenetically informative *trnT-F* gene region have led to a more resolved phylogenetic tree than that of Levin & al. (2006). We recognize 14 major clades in the spiny solanums. These clades remain informally named, as further re-circumscriptions of sections within the Leptostemonum clade should await exhaustive, clade by clade studies to avoid further confusion of infrageneric taxonomy. We provide descriptions of the major clades below with details of their geographical distribution, morphological characters, and, when possible, estimates of expected species numbers. Provisional species lists for these clades can be found on the Solanaceae Source webpage (www.solanaceaesource.org).

Of particular importance in this study is the division of the Robustum clade of Levin & al. (2006) into the Erythrotrichum, Thomasiifolium, and Gardneri clades. Their study suggested two strongly supported clades within the Robustum clade, one containing *S. accrescens*, *S. robustum*, and *S. stagnale* and the other containing *S. agrarium*, *S. stenandrum*, and *S. microphyllum*. Our increased sampling confirms the presence of two well-supported clades within the Robustum clade as suggested by Levin & al. (2006), here denoted as the Erythrotrichum and Gardneri clades. Sampling of taxa not included in Levin & al. (2006) indicates the presence of a third clade, the Thomasiifolium clade.

Our study also strengthens the sister relationship of the Acanthophora and Lasiocarpa clades that was weakly supported in Levin & al. (2006). Finally, our study suggests that the Androceras/Crinitum clade is sister to the Sisymbriifolium clade. While these results help to clarify the relationships within the Leptostemonum clade, there remains a largely unresolved group that involves many of the most speciose clades, including the large Torva clade and the very diverse Old World clade. Further study with more molecular data is underway in an attempt to resolve relationships in this portion of the tree (S. Stern & L. Bohs, unpub. data).

Torva clade. — We have greatly increased the sampling of the Torva clade from the 5 species used in Levin & al. (2006) to 25 species. The Torva clade corresponds to Whalen's (1984) *S. torvum* species group and Nee's (1999) sect. *Torva* Nees, which are concordant in circumscription and contain approximately 45 species. The Torva clade is consistent with Whalen and Nee's concepts but also includes *S. poinsettiifolium* and *S. subinerme*. Whalen (1984) placed *S. subinerme* in his *S. subinerme* species group and did not treat *S. poinsettiifolium*. Nee (1999) placed the two species together in sect. *Micracantha* Dunal.

Members of the Torva clade are scandent to erect shrubs or small trees with usually branched inflorescences, straight or occasionally recurved spines, stellate corollas with narrow triangular lobes and often abundant interpetalar tissue, and small to medium-sized leathery berries with mucilaginous pulp. The Torva clade is found throughout the Neotropics but is most diverse in the Andes and is characteristic of secondary vegetation and grazed land, with some species, most notably *S. torvum*, being noxious weeds in the Old World. *Solanum poinsettiifolium* and *S. subinerme* have recurved prickles, simple inflorescences, and curved buds and anthers, leading Whalen (1984) and Nee (1999) to exclude them from the *S. torvum* species group and sect. *Torva*, respectively, though both authors noted their probable affinities with these groups. These species have narrowly triangular corolla lobes and leathery mucilaginous berries typical of the Torva clade and are clearly placed within it on the basis of molecular data.

Within the Torva clade there is little bootstrap support for many of the higher-level nodes; however, many of the sister species relationships are strongly supported. Clarification of the relationships in this group will undoubtedly require the use of more molecular markers.

Micracantha clade. — Nee (1999) placed 19 species within sect. *Micracantha.* We sampled 13 of these species, and 9 of these fall within the Micracantha clade, corresponding to Nee's (1999) subsection 1 series 1 and 3 and subsection 3. In contrast, Whalen (1984) included 11 species in his *S. lanceifolium* species group, and, accounting for changes in nomenclature, we sampled all of these. The species composition of the Micracantha clade recovered in our analysis is more similar to Whalen's (1984) concept of the group, with the inclusion of *S. jamaicense*, which he left as an unplaced species. Both classifications focused on the fact that the plants climb via recurved prickles; however, as noted below, these characteristics appear to have evolved multiple times.

The Micracantha clade is native to the Neotropics, ranging from south Florida through the Caribbean and Central America and tropical South America to Bolivia. Members of the clade are typically found in disturbed habitats, including river banks, swamps, and forest gaps. They have convergent traits with other groups in the Leptostemonum clade, including a viny habit and recurved prickles found in some species of the Torva clade (*S. subinerme*, *S. poinsettiifolium*), the Crinitum clade (*S. coriaceum* and *S. sendtnerianum*), and the Thomasiifolium clade (*S. paraibanum*, *S. rupincola*). Despite this convergence, members of this clade are always vines or scandent shrubs with unbranched inflorescences, strictly recurved spines, and deeply stellate corollas with strap-shaped lobes and very little interpetalar tissue. A revision of the Micracantha clade along with further phylogenetic study using additional molecular markers is clarifying the relationships within this group (S. Stern, unpub. data).

Carolinense clade. — The composition of the Carolinense clade is similar to that of Levin & al. (2006), with the addition of *S. juvenale* and *S. moxosense*. It is composed of small rhizomatous, erect or prostrate plants with abundant needle-like prickles, usually densely spiny calyces (except in *S. moxosense*), an andromonoecious reproductive system, and small to medium-sized, green to yellow mottled fruits. The group has a disjunct geographic range, with *S. carolinense* native to the southeastern United States and the other members native to arid areas of Paraguay, Argentina, and Bolivia.

Whalen (1984) placed the South American species *S. aridum* (as the synonym *S. conditum* C.V. Morton), *S. juvenale*, and *S. comptum* in his *S. multispinum* species group but kept the North American *S. carolinense* as unplaced. Nee (1999) placed *S. aridum*, *S. juvenale*, *S. comptum*, and *S. carolinense* in his sect. *Melongena* (Mill.) Dunal subsect. *Lathyrocarpum* G. Don; however, he also included *S. hasslerianum*, which our analysis places in the Sisymbriifolium clade and *S. mortonii*, which our analysis places in the Elaeagnifolium clade. Neither classification included *S. moxosense*, a Bolivian species described more recently (Nee & al., 2006). *Solanum hieronymi* and *S. multispinum* were placed with members of the Carolinense clade in Nee's (1999) sect. *Melongena* and Whalen's (1984) *S. multispinum* group due to their similarities in habitat, distribution, and morphology with species of this clade. However, our results are similar to those of Levin & al. (2006) indicating that neither *S. hieronymi* nor *S. multispinum* are supported as members of the Carolinense clade (discussed below).

Asterophorum clade. — The two representatives of the Asterophorum clade have not previously been included in phylogenetic studies. The species in this clade are native to northeastern (*S. asterophorum*) and southeastern Brazil (*S. piluliferum*) and characterized by pronounced zig-zag stems with paired and unequal leaves, small leaf-opposed inflorescences, and accrescent fruiting calyces.

The strong support for *S. asterophorum* and *S. piluliferum* as sister taxa corresponds to Whalen's (1984) *S. asterophorum* species group, which contained only these two species. Nee (1999) did not treat *S. piluliferum*, but these data refute his placement of *S. asterophorum* in sect. *Polytrichum*, as our molecular data place *S. polytrichum* in the Gardneri clade.

Bahamense clade. — Similar to the results of Levin & al. (2006), the two species in the Bahamense Clade, *S. bahamense* and *S. ensifolium* (previously referred to as *S. drymophilum* O.E. Schulz), are strongly supported as a monophyletic group. These species are found in the Caribbean, and a detailed study of the Bahamense species group has shown that it contains an additional species, *S. polyacanthos* Lam., native to Hispaniola (Strickland-Constable & al., 2010). Although there are striking morphological differences within this group, most notably in the diminutive leaves, flowers, and fruits of *S. polyacanthos*, these species are united by their scattered needle-like prickles, deeply stellate corollas, small juicy red or black fruits on strongly recurved fruiting pedicels, and the presence of stellate hairs on the adaxial surface of the anthers, an unusual characteristic within the spiny solanums (Whalen, 1984; Strickland-Constable & al., 2010).

Whalen's (1984) *S. bahamense* species group contained six species native to the West Indies, including *S. bahamense*, *S. ensifolium*, and *S. polyacanthos*; the other three species he included are now regarded as synonyms of *S. bahamense* or *S. ensifolium* (Strickland-Constable & al., 2010). Nee (1999) treated *S. ensifolium* as a synonym of *S. bahamense* and placed it in sect. *Persicariae*, along with 14 other species. Aside from *S. bahamense*, our analysis sampled six of the species of this section and placed them in at least two different clades. Our results indicate *S. gardneri*, S. *microphyllum*, *S. schomburghii* and *S. tetramerum* are part of the Gardneri clade, *S. buddleifolium* is in the Thomasiifolium clade, and *S. crotonoides* is unassigned among the 14 clades.

Elaeagnifolium clade. — The Elaeagnifolium clade is the same as that of Levin & al. (2006) with the addition of *S. mortonii* that they did not sample. Members of this clade are rhizomatous herbs with dense, almost felt-like pubescence, purple corollas, and somewhat accrescent fruiting calyces. Species of the Elaeagnifolium clade show pronounced floral dimorphism; *S. elaeagnifolium*, *S. mortonii*, and *S. hindsianum* have flowers that are actinomorphic or nearly so, whereas those of *S. tridynamum* are zygomorphic, with three long lower and two short upper stamens in the staminate flowers (Bohs & al., 2007). *Solanum hindsianum* and *S. tridynamum* have an unusual fruit type that is erect, dry, and circumsessile or irregularly dehiscent, with the seeds dispersed passively by wind or rain action (Lester & Symon, 1989; Knapp, 2002).

Members of this clade share morphological characteristics with the Carolinense clade, such as needle-like spines, small to medium-sized yellow or greenish fruits, and an andromonoecious reproductive system in those species that have been studied, including *S. elaeagnifolium* (Martine & al., 2009), *S. mortonii* (Hunziker, 1979), and *S. tridynamum* (Bohs & al., 2007). This morphological convergence is likely due to the clades inhabiting similar arid habitats in North and South America. Like the Carolinense clade, the Elaeagnifolium clade has a large geographical disjunction, with some members found in the United States and Mexico (*S. hindsianum*, *S. tridynamum*), others in South America (*S. mortonii*), and with *S. elaeagnifolium* found in both areas.

Whalen (1984) placed *S. elaeagnifolium* in the *S. ellipticum* species group and *S. tridynamum* in the *S. vespertilio* species group. However, these groups both contained species from the Old and New World and are clearly not monophyletic since nearly all of the Old World spiny solanums form a clade (see below). Whalen left *S. hindisanum* unplaced and did not treat *S. mortonii*. Nee (1999) placed the members of the Elaeagnifolium clade in sect. *Melongena* subsect. *Lathyrocarpum*. However, this subsection also contained species that our results place in different clades, such as *S. aridum*, *S. comptum*, and *S. juvenale* (Carolinense clade), *S. hasslerianum* (Sisymbriifolium clade), and *S. hieronymi* and *S. multispinum*, which are unassigned to any of the 14 clades.

Old World clade. — The four representatives of the Old World clade included here are from Africa, Asia, and Australia, and form part of a much larger strongly supported monophyletic group that includes the majority of Old World spiny solanums (Levin & al., 2006; Weese & Bohs, 2007, 2010). This clade represents a large radiation with a wide range of phenotypes. No single or combination of morphological characters appears to define this group. The main feature defining this clade is its geographical location, as very few species of Old World spiny solanums fall outside of the Old World clade. A few notable exceptions, such as *S. torvum* of the Torva clade are introductions from the New World, whereas a few taxa such as *S. lasiocarpum* and *S. repandum* of the Lasiocarpa clade apparently represent independent radiations into the Old World from New World ancestors. Detailed study of the Old World spiny solanums is underway and will help to clarify relationships within this clade (M. Vorontsova & al., unpub. data; L. Bohs & al., unpub. data).

Androceras/Crinitum clade. — Species placed in Whalen's (1984) *S. rostratum* species group and Nee's (1999) sect. *Androceras* (Nutt.) Whalen (*S. citrullifolium*, *S. rostratum*, *S. grayi*) form a strongly supported monophyletic group nested within a larger clade of species placed in Whalen's (1984) *S. crinitum* species group and Nee's (1999) sect. *Crinitum* (Whalen) Child (*S. urticans*, *S. wrightii*, *S. crinitum*, *S. lycocarpum*). Our data also support the inclusion of the Amazonian *S. coriaceum* and *S. sendtnerianum* as well as the Mexican *S. mitlense* in the Androceras/Crinitum clade. While Whalen (1984) did not treat either *S. coriaceum* or *S. sendtnerianum*, Nee (1999) placed these species in sect. *Micracantha* based on their viny habit. However, other morphological characters, including large flowers and fruits with swellings on the fruiting calyx, support the relationship of these taxa with sect. *Crinitum*. Whalen (1984) left *S. mitlense* as an unplaced taxon but suggested it might be related to the *S. torvum* species group, whereas Nee (1999) tentatively placed it within sect. *Crinitum*, also suggesting that it might be a derived member of sect. *Torva*. These placements were likely due to the disjunct distribution of *S. mitlense* in Mexico where many species of sect. *Torva* are common, whereas the other species in sect. *Crinitum* are restricted to South America. The morphological characters of *S. mitlense*, including habit and flower, fruit, and seed size, are all typical of sect. *Crinitum* (F. Farruggia, unpub. data).

Although the parsimony strict consensus tree (Fig. 1) did not resolve the Crinitum group as monophyletic, the Bayesian analysis of our dataset (not shown) resulted in a monophyletic Androceras clade sister to a monophyletic Crinitum clade; however, the latter had low support (0.70 PP). A more detailed study of sect. *Androceras* (Stern & al., 2010) showed that the section is monophyletic and sister to a monophyletic sect. *Crinitum*, which includes *S. mitlense* and *S. sendtnerianum* (*S. coriaceum* was not sampled). This difference in our parsimony analysis and the result of Stern & al. (2010) is likely due to the exhaustive sampling from sect. *Androceras* in the latter study, whereas here only three placeholder taxa were included from the approximately 12 species in the section. *Solanum* sect. *Crinitum* is under further morphological and molecular study (F. Farruggia, unpub. data) to examine its monophyly and the phylogenetic relationships among its component species.

Sections *Androceras* and *Crinitum* have distinct morphologies and geographic ranges, and it is expected that further studies with broader taxonomic sampling and additional molecular markers will support each as a monophyletic group. Members of sect. *Androceras* are among the most recognizable of the spiny solanums because of their distinctive zygomorphic, heterantherous, enantiostylous flowers, dry fruits with a persistent, prickly, and accrescent calyx, and their primarily north temperate distribution (Stern & al., 2010). This contrasts with sect. *Crinitum*, which is composed of large shrubs, trees, or woody vines with some of the largest flowers and fruits in the genus. Members of sect. *Crinitum* also have unique swellings on the fruiting calyx, fruits that oxidize black when cut open, and a center of diversity in tropical South America.

Sisymbriifolium clade. — The Sisymbriifolium clade contains two species, *S. sisymbriifolium* and *S. hasslerianum*, that have not previously been placed together. Both are found in Paraguay and northern Argentina but *S. sisymbriifolium* also has a broader distribution through the Andes into northern South America. These species are robust herbs or small shrubs with deeply pinnately lobed leaves and spiny, accrescent fruiting calyces. Whalen (1984) left *S. sisymbriifolium* unplaced, and he included *S. hasslerianum* in the *S. polytrichum* species group along with *S. hexandrum*, *S. polytrichum*, *S. stagnale*, and *S. urticans* that were sampled here. Our results show that the latter four species belong to three different clades, and none appear closely related to *S. hasslerianum*. Nee (1999) placed *S. sisymbriifolium* and *S. hasslerianum* in separate subsections within his sect. *Melongena*, each associated with species that emerge in other clades in the molecular phylogenies. Evidently these placements were based on convergent morphological characters and do not reflect phylogenetic relatedness.

Acanthophora and Lasiocarpa clades. — The Acanthophora clade largely corresponds to Whalen's (1984) *S. mammosum* species group and Nee's (1999) sect. *Acanthophora* Dunal, which was monographed by Nee (1979). Levin & al. (2005) showed that *S. agrarium* and *S. stenandrum*, which had traditionally been placed in sect. *Acanthophora*, clearly do not belong within this group. Our data support this exclusion but otherwise support the traditional circumscription of sect. *Acanthophora*. The Lasiocarpa clade corresponds to the *S. quitoense* species group of Whalen (1984) and Nee's (1999) sect. *Lasiocarpa* (Dunal) D'Arcy which was taxonomically revised by Whalen & al. (1981). Bohs (2004) and our data both support the monophyly of the Lasiocarpa clade.

The Acanthophora and Lasiocarpa clades are both native to the Neotropics, with a few species in the Lasiocarpa clade, including *S. ferox* L., *S. lasiocarpum*, and *S. repandum* G. Forst., and perhaps one species of the Acanthophora clade, *S. aculeatissimum* Jacq., native in the Old World (Bohs, 2004; Levin & al., 2005). The Acanthophora clade includes some of the most vicious examples of the spiny solanums, with needle-like prickles found throughout the plant and the unusual feature of exclusively simple hairs on the upper leaf surfaces in most species (Nee, 1979). The Lasiocarpa clade can be distinguished by its large, shallowly lobed leaves, unbranched infloresences, and typically large fruits covered with stellate hairs (Whalen & al., 1981).

We chose to sample a few representative species from each clade because they were well-sampled in Levin & al. (2006), building on phylogenetic work by Bohs (2004) on the Lasiocarpa clade and Levin & al. (2005) for the Acanthophora clade. These clades were found to be sister to each other in Levin & al. (2006), but with low support (64% BS). Our analysis strongly supports the Lasiocarpa clade as sister to the Acanthophora clade (85% BS, 1.0 PP). We included two additional species not previously sampled, *S. vaillantii* and an undescribed Brazilian species which our data place in the Acanthophora clade. Whalen (1984) and Nee (1999) placed *S. vaillantii* in their *S. mammosum* species group and sect. *Acanthophora*, respectively. These groups correspond to our Acanthophora clade. The undescribed species matches the description given for *Solanum* sp. 1 in Mentz & Oliveira (2004) and a named but unpublished species included in Nee (1979). It is from southeastern Brazil and has morphological characters that are typical of the Acanthophora clade, including needle-like prickles and simple hairs on the upper leaf surfaces.

Erythrotrichum clade. —The Erythrotrichum clade is distinguished from other spiny solanum groups by its plurifoliate sympodial units, the presence of recurved prickles, a ferruginous to reddish tomentum with stellate-glandular trichomes, an andromonoecious reproductive system, and pubescent fruits with large seeds (Agra, 2008). There appear to be three centers of diversity for this group: Central America, northeastern Brazil, and the Andes of Peru and Ecuador.

As defined here, the Erythrotrichum clade is morphologically homogeneous and largely congruent with Nee's (1999) sect. *Erythrotrichum* (Whalen) Child, which included *S. accrescens*, *S. megalonyx*, *S. paludosum*, and *S. robustum*, and Whalen's (1984) *S. erythrotrichum* species group, which included *S. accrescens*, *S. decompositiflorum*, *S. decorum*, *S. megalonyx*, and *S. robustum*. Whalen (1984) did not include *S. paludosum*, *S. reflexiflorum*, and *S. rhytidoandrum* in his treatment, while Nee (1999) did not treat *S. decorum*, *S. decompositiflorum*, *S. reflexiflorum*, and *S. rhytidoandrum*. In more recent treatments, Agra (2004, 2007, 2008) listed all of these species as members of sect. *Erythrotrichum* except *S. reflexiflorum*, which she left as a species of uncertain placement; however, *S. robustum* was not included in her revision of sect. *Erythrotrichum* (Agra, 2004) or her key to the section (Agra, 2008). The Erythrotrichum clade also includes *S. absconditum* and *S. jabrense* that were described by Agra (2004, 2008) after the publications of Whalen (1984) and Nee (1999) and considered by her to belong to sect. *Erythrotrichum*.

Levin & al. (2006) sampled three species from the Erythrotrichum clade (*S. accrescens*, *S. robustum*, *S. stagnale*) and found them to form a well-supported monophyletic group included within their Robustum clade. Our increased sampling, now encompassing 9 of the 22 species included in sect. *Erythrotrichum* by Agra (2008), reveals that they all form a well-supported monophyletic group that also includes *S. hexandrum*, *S. reflexiflorum*, *S. robustum*, and *S. stagnale* that Agra (2008) did not treat. *Solanum hexandrum* and *S. stagnale* have not been included in sect. *Erythrotrichum* by previous authors. Whalen (1984) and Nee (1999) included both species in the *S. polytrichum* species group and sect. *Polytrichum*, respectively; Agra (2007) included *S. hexandrum* in sect. *Polytrichum* but left *S. stagnale* unplaced. According to our results, these species clearly belong to the Erythrotrichum clade. Within the Erythrotrichum clade, *S. hexandrum*, *S. robustum*, and *S. stagnale* form a monophyletic group, but with poor bootstrap support. The three species are morphologically similar, with large decurrent leaf blades and accrescent fruiting calyces. *Solanum reflexiflorum* was not treated by Whalen (1984) or Nee (1999), and Agra (2007) left it unplaced. This species, endemic to the state of Bahia, Brazil, has reduced infloresences, small flowers with reflexed calyx lobes, and glabrous fruits, but is evidently a member of the Erythrotrichum clade according to molecular data.

Thomasiifolium clade. — The Thomasiifolium clade is difficult to define morphologically, as it contains two seemingly distinct groups, both found in eastern Brazil. These include the shrubby to scandent *S. thomasiifolium* and *S. buddleifolium*, and the vines *S. paraibanum* and *S. rupincola*. All have plurifoliate sympodial units and pale lilac to blue or violet flowers with somewhat coriaceous corollas. The latter two climb using recurved prickles and have large, densely pubescent fruits with large seeds. The shrubs *S. thomasiifolium* and *S. buddleifolium* have straight spines and smaller, glabrous fruits with smaller seeds.

The Thomasiifolium clade includes species from Whalen's (1984) *S. polytrichum* species group (*S. rupincola*), taxa he left unplaced (*S. buddleifolium*, *S. thomasiifolium*), and *S. paraibanum*, which was described after his conspectus was published (Agra, 1992). Nee (1999) placed *S. buddleifolium* in sect. *Persicariae*, *S. paraibanum* in sect. *Micracantha*, and did not treat *S. rupincola*. He tentatively placed *S. thomasiifolium* in both sections *Erythrotrichum* and *Persicariae* because it shares morphological characteristics of both sections.

We have chosen to separate the Thomasiifolium clade from its sister group, the Erythrotrichum clade, due to its moderate support as a monophyletic and morphologically distinct group. This clade is in need of more morphological and molecular study and additional field work, and further study may show that it should be broken into two or more discrete clades.

Gardneri clade. — Members of the Gardneri clade are native from eastern to central Brazil (*S. agrarium*, *S. gardneri*, *S. polytrichum*, *S. stenandrum*) or the Caribbean (*S. microphyllum*, *S. schomburghii*, *S. tetramerum*) with one species from northern Peru (*S. talarense*). Defining the Gardneri clade morphologically is difficult. Possible uniting characteristics include a slender-stemmed, herbaceous to shrubby habit, straight, often needle-like prickles, small leaves in many species, short, laterally directed inflorescences, somewhat accrescent fruiting calyces, and preferences for arid habitats.

Species in the Gardneri clade come from multiple groups of previous classifications. Whalen (1984) left *S. gardneri*, *S. microphyllum*, and *S. tetramerum* (as the synonym *S. aquartia* Dunal) as unplaced species, although he noted that the latter two were probably related to each other. He placed *S. polytrichum* in the *S. polytrichum* species group, *S. stenandrum* and *S. agrarium* in the *S. mammosum* species group, and did not treat *S. schomburghii* or *S. talarense*. Nee (1999) also placed the species in disparate groups, with *S. gardneri*, *S. microphyllum*, *S. schomburghii*, and *S. tetramerum* (as *S. aculeatum* (Jacq.) O.E. Schulz) in sect. *Persicariae*, *S. polytrichum* in sect. *Polytrichum*, and *S. stenandrum*, *S. agrarium*, and *S. talarense* in sect. *Acanthophora*. The Gardneri clade also includes an undescribed species from Brazil.

Extensive sampling of Whalen's (1984) *S. mammosum* species group and Nee's (1999) sect. *Acanthophora* by Levin & al. (2005) revealed that *S. stenandrum* and *S. agrarium* lie outside the Acanthophora clade, despite the presence of mainly simple hairs on the adaxial leaf surfaces, a hallmark of the group. Levin & al. (2006) obtained this same result in their broader study of the spiny solanums; *S. stenandrum* and *S. agrarium* did not belong to the Acanthophora clade, but instead formed a well-supported group with *S. microphyllum* that was included as part of their Robustum clade. With our increased taxonomic sampling, we now recognize three clades, the Erythrotrichum, Thomasiifolium, and Gardneri clades, within the Robustum clade of Levin & al. (2006).

Solanum stenandrum and *S. agrarium* are morphologically similar to *S. talarense* from northern Peru and an undescribed species from the planalto of central Brazil. All share exclusively or predominantly simple and often glandular pubescence on the adaxial leaf surfaces, small leaves, and few-flowered inflorescences with white or greenish yellow corollas. All except *S. stenandrum* have a largely prostrate growth habit and all four species are adapted to arid habitats.

The remaining species of the Gardneri clade are a somewhat heterogeneous assemblage. Two species, *S. microphyllum* and *S. tetramerum*, have similar morphologies, including densely pubescent, reduced leaves, likely due to their similar habitats in xeric areas of the Caribbean. *Solanum schomburghii* and *S. gardneri* have similar appearances due to their dense, white stellate hairs and very long, tapering anthers. Finally, *S. polytrichum* is very distinctive due to its long, bristly pubescence and the prickly calyces that nearly completely cover the fruit. More sampling from eastern Brazil and the Caribbean will improve our phylogenetic understanding of the Gardneri clade. However, current results are sufficient to state that neither sect. *Polytrichum* nor sect. *Persicariae* as circumscribed by Nee (1999) are monophyletic.

Taxa unassigned among the 14 clades. — Despite broader sampling, four taxa, *S. campechiense*, *S. crotonoides*, *S. hieronymi*, and *S. multispinum* remain unassigned among the 14 clades recognized here. These are all taxa that also were unplaced in Levin & al. (2006). *Solanum campechiense*, a species from marshy areas of Central America, the Caribbean, and northeastern South America, continues to be an enigmatic species. Its sequences are highly divergent and it has distinctive morphological characteristics including a sprawling habit, abundant straight prickles, deeply lobed leaves, and green, yellow, or purplish fruits surrounded by accrescent spiny calyces. Both Nee (1999) and Whalen (1984) suggested a relationship between *S. campechiense* and *S. sisymbriifolium*, but this relationship is not supported by our data. *Solanum crotonoides* is known only from montane forests of Hispaniola and is unusual in the spiny solanums in its dioecious reproductive system and strongly discolorous leaves due to almost lepidote pubescence on the abaxial surfaces. Nee (1999) placed *S. crotonoides* in sect. *Persicariae*, which is clearly not monophyletic (see discussion under Gardneri clade). Whalen (1984) placed *S. crotonoides* in the *S. crotonoides* species group along with three other species from the Caribbean, none of which were sampled in this study. Sampling these and other Caribbean species is key for understanding its phylogenetic affinities. As in Levin & al. (2006), *S. hieronymi* is supported as sister to the Elaeagnifolium+Old World clades. It is found in Argentina, Bolivia, and Paraguay and has morphological similarities with the Elaeagnifolium clade, including a rhizomatous, herbaceous habit, needle-like spines, and a densely, spiny accrescent calyx. Finally, *S. multispinum* also remains unplaced. It is found in Argentina, Paraguay, and adjacent regions of Brazil and is a small, rhizomatous herb with abundant needle-like prickles, an andromonoecious reproductive system, and mottled, yellow-green fruits. Its morphology and distribution point to it being related to members of the Carolinense clade; however, molecular data are, at present, inconclusive as to its relationships.

Steps to a systematic revision of the "spiny solanums". — A major contribution of this study is the identification of 14 major well-supported clades within the spiny solanums, using many New World taxa sampled here for the first time. In many cases, these clades are defined by unique morphological features and/or geographical ranges. Future studies are underway to resolve some of the remaining polytomies and ambiguously placed taxa using newly developed COSII markers (Wu & al., 2006; Rodriguez & al., 2009) and additional chloroplast markers (Shaw & al., 2007; Miller & al., 2009). Further taxonomic sampling, particularly from Brazilian and Caribbean species, is likely to identify new clades within the spiny solanums and clarify relationships within the Asterophorum, Gardneri, Thomasiifolium, and Erythrotrichum clades. More detailed studies of specific clades, particularly the Old World clade and *S. crinitum* species group, are in progress using much greater sampling density than that reported here. These molecular analyses, in concert with morphological studies, should lead us in the near future to a detailed understanding of evolutionary relationships in the large, complex, and widely distributed Leptostemonum clade.

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■ LITERATURE CITED

Agra, M.F. 1992. A new species of *Solanum* section *Micracantha* (Solanaceae) from northeastern Brazil. *Novon* 2: 179–181.

Agra, M.F. 2004. Sinopse taxonômica de *Solanum* sect. *Erythrotrichum* (Solanaceae). Pp. 192–211 in: Rangel-Ch., J.O., Aguirre, C.J., Andrade-C., M.G. & Cañas, D.G. (eds.), *Memorias Octavo Congresso Latinoamericano e Segundo Colombiano de Botánica*. Bogotá: Universidad Nacional de Colombia.

Agra, M.F. 2007. Diversity and distribution of *Solanum* subg. *Leptostemonum* in Brazil. *Acta Hort.* 745: 31–42.

Agra, M.F. 2008. Four new species of *Solanum* section *Erythrotrichum* (Solanaceae) from Brazil and Peru, and a key to the species of the section. *Syst. Bot.* 33: 556–565.

Bohs, L. 2004. A chloroplast DNA phylogeny of *Solanum* section *Lasiocarpa*. *Syst. Bot.* 29: 177–187.

Bohs, L. 2005. Major clades in *Solanum* based on *ndhF* sequence data. Pp. 27–49 in: Keating, R.C., Hollowell, V.C. & Croat, T.B. (eds.), *A festschrift for William G. D'Arcy: The legacy of a taxonomist*. St. Louis: Missouri Botanical Garden Press.

Bohs, L. & Olmstead, R.G. 2001. A reassessment of *Normania* and *Triguera* (Solanaceae). *Pl. Syst. Evol.* 228: 33–48.

Bohs, L., Weese, T., Myers, N., Lefgren, V., Thomas, N., Van Wagenen, A. & Stern, S. 2007. Zygomorphy and heterandry in *Solanum* in a phylogenetic context. *Acta Hort.* 745: 201–223.

Cummings, M.P., Handley, S.A., Myers, D.S., Reed, D.L., Rokas, A. & Winka, K. 2003. Comparing bootstrap and posterior probability values in the four-taxon case. *Syst. Biol.* 52: 477–487.

D'Arcy, W.G. 1972. Solanaceae studies II: Typification of subdivisions of *Solanum*. *Ann. Missouri Bot. Gard.* 59: 262–278.

Erixon, P., Svennblad, B., Britton, T. & Oxelman, B. 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Syst. Biol.* 52: 665–673.

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

Frodin, D.G. 2004. History and concepts of big plant genera. *Taxon* 53: 753–776.

Goloboff, P., Farris, J. & Nixon, K. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24: 774–786.

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Hunziker, A.T. 1979. Estudios sobre Solanaceae. IX. *Solanum mortonii*, especie nueva del noroeste de Argentina. *Kurtziana* 12–13: 133–138.

Knapp, S. 2002. Tobacco to tomatoes: A phylogenetic perspective on fruit diversity in the Solanaceae. *J. Exp. Bot.* 53: 2001–2022.

Knapp, S., Bohs, L., Nee, M. & Spooner, D.M. 2004. Solanaceae – A model for linking genomics with biodiversity. *Comp. Funct. Genomics* 5: 285–291.

Lester, R.N. & Symon, D.E. 1989. A Mexican *Solanum* with splash-cup or censer fruits. *Solanaceae Newsl.* 3(1): 72–73.

Levin, R.A., Myers, N.R. & Bohs, L. 2006. Phylogenetic relationships among the "spiny solanums" (*Solanum* subgenus *Leptostemonum*, Solanaceae). *Amer. J. Bot.* 93: 157–169.

Levin, R.A., Watson, K. & Bohs, L. 2005. A four-gene study of evolutionary relationships in *Solanum* section *Acanthophora*. *Amer. J. Bot.* 92: 603–612.

Martine, C.T., Anderson, G.J. & Les, D.H. 2009. Gender-bending aubergines: Molecular phylogenetics of cryptically dioecious *Solanum* in Australia. *Austral. Syst. Bot.* 22: 107–120.

Martine, C.T., Vanderpool, D., Anderson, G.J. & Les, D.H. 2006. Phylogenetic relationships of andromonoecious and dioecious Australian species of *Solanum* subgenus *Leptostemonum* section *Melongena*: Inferences from ITS sequence data. *Syst. Bot.* 31: 410–420.

Mentz, L.A. & Oliveira, P.L. 2004. *Solanum* (Solanaceae) na região sul do Brasil. *Pesq., Bot.* 54: 1–327.

Miller, J.S., Kamath, A. & Levin, R.A. 2009. Do multiple tortoises equal a hare? The utility of nine noncoding plastid regions for species-level phylogenetics in tribe Lycieae (Solananceae). *Syst. Bot.* 34: 796–804.

Nee, M. 1979. A revision of *Solanum* section *Acanthophora*. Ph.D. dissertation, University of Wisconsin, Madison, Wisconsin, U.S.A.

Nee, M. 1999. Synopsis of *Solanum* in the New World. Pp. 285–333 in: Nee M., Symon D.E., Lester R.N. & Jesops J.P. (eds.), *Solanaceae IV: Advances in biology and utilization*. Kew: Royal Botanic Gardens.

Nee, M., Knapp, S. & Bohs, L. 2006. New species of *Solanum* and *Capsicum* (Solanaceae) from Bolivia, with clarification of nomenclature in some Bolivian *Solanum*. *Brittonia* 58: 322–356.

Posada, D. & Crandall, K.A. 1998. MODELTEST: Testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Rambaut, A. 1996. Se-Al: Sequence alignment editor. http://evolve.zoo.ox.ac.uk/. Department of Zoology, University of Oxford, Oxford, U. K.

Rodríguez, F., Wu, F., Ané, C., Tanksley, S. & Spooner, D.M. 2009. Do potatoes and tomatoes have a single evolutionary history, and what proportion of the genome supports this history? *B.M.C. Evol. Biol.* 9: 191. DOI: 10.1186/1471-2148-9-191.

Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *Amer. J. Bot.* 94: 275–288.

Simmons, M.P., Pickett, K.M. & Miya, M. 2004. How meaningful are Bayesian support values? *Molec. Biol. Evol*. 21: 188–199.

Stern, S., Weese, T. & Bohs, L. 2010. A three-gene phylogeny of *Solanum* section *Androceras*. *Syst. Bot.* 33: 885–893.

Strickland-Constable, R., Schneider, H., Ansell, S.W., Russell, S.J. & Knapp, S. 2010. Species identity in the *Solanum bahamense* species group (Solanaceae, *Solanum* subgenus *Leptostemonum*). *Taxon* 59: 209–226.

Swofford, D.L. 2002. PAUP*: Phylogenetic analysis using parsimony (* and other methods), version 4.0b10. Sunderland, Massachusetts: Sinauer.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1110.

Weese, T.L. & Bohs, L. 2007. A three-gene phylogeny of the genus *Solanum* (Solanaceae). *Syst. Bot*. 32: 445–463.

Weese, T.L. & Bohs, L. 2010. Eggplant origins: Out of Africa, into the Orient. *Taxon* 59: 49–56.

Whalen, M.D. 1984. Conspectus of species groups in *Solanum* subgenus *Leptostemonum*. *Gentes Herb.* 12: 179–282.

Whalen, M.D., Costich, D.E. & Heiser, C.B. 1981. Taxonomy of *Solanum* section *Lasiocarpa*. *Gentes Herb.* 12: 41–129.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in: Innis, M., Gelfand, D., Sninsky, J. & White, T. (eds.), *PCR protocols: A guide to methods and applications*. San Diego: Academic Press.

Wiens, J.J. 1998. Combining data sets with different phylogenetic histories. *Syst. Biol.* 47: 568–581.

Wu, F., Mueller, L.A., Crouzillat, D., Pétiard, V. & Tanksley, S.D. 2006. Combining bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes (COSII) for comparative, evolutionary and systematic studies: A test case in the Euasterid plant clade. *Genetics* 174: 1407–1420.

Appendix. Summary of species, collection location, vouchers, herbarium acronym, and GenBank accession numbers for taxa used in this study provided in the order ITS, *waxy*, and *trnT-F.* Dashed lines indicate that the region was not sampled for this accession. NIJ accession numbers refer to the Solanaceae collection at Radboud University, Nijmegen, the Netherlands. BIRM samples have the seed accession number for the Solanaceae collection at the University of Birmingham, U.K.

Solanum absconditum Agra, Brazil, *Agra & al. 7021* (JPB); GU591053, GU591105, GU590994. ***S. abutiloides*** (Griseb.) Bitter & Lillo, BIRM S.0655, *Olmstead S-73* (WTU); AF244716, AY562948, AY266236. **S. *accrescens*** Standl. & C.V. Morton, Costa Rica, *Bohs 2556* (UT); AY996480, AY996375, DQ180473. ***S. acerifolium*** Dunal, Costa Rica, *Bohs 2714* (UT); AY561261, AY562949, AY266149. ***S. acutilobum*** Dunal, Bolivia, *Bohs 2897* (UT); GU591054, GU591106, GU590995. ***S. adhaerens*** Roem. & Schult., Costa Rica, *Bohs 2473* (UT); AF244723, AY996377, DQ180474. ***S. aethiopicum*** L., BIRM S.0344, *Olmstead S-74* (WTU); AY996482**,** AY996378**,** DQ180394**.** ***S. agrarium*** Sendtn., Brazil, *Agra & al. 7027* (JPB); GU591055, GU591107, GU590996. ***S. albidum*** Dunal, Bolivia, *Nee 51831* (NY); GU591056, GU591108, GU590997. ***S. alternatopinnatum*** Steud., Brazil, *Agra & al. 7227* (JPB); HQ457395, HQ457414, HQ457404. ***S. arachnidanthum*** Rusby, Bolivia, *McClelland & Stern 412* (NY); GU591057, GU591109, GU590998. ***S. arboreum*** Dunal, Costa Rica, *Bohs 2521* (UT); AF244719, AY996381, DQ180424. ***S. argentinum*** Bitter & Lillo, Argentina, seeds from *Zygadlo 100, Bohs 2539* (UT); AF244718, AY996382, DQ180425. ***S. aridum*** Morong, Bolivia, *Bohs & Nee 2733* (UT); AY996499, AY996400, DQ180479**.** ***S. asperolanatum*** Ruiz & Pav., Bolivia, *Nee 51761* (UT); GU591058, GU591110, GU590999. ***S. asterophorum*** Mart., Brazil, *Agra & al. 7010* (JPB); GU591059, GU591111, GU591000. ***S. asteropilodes*** Bitter, Ecuador, *Bohs 3445* (UT); GU591060, GU591112, GU591001. ***S. aturense*** Dunal, Costa Rica, *Bohs 2976* (UT); AY996486, AY996385, GU591002. ***S. bahamense*** L., NIJ 944750187, *Bohs 2936* (UT); AY996487, AY996386, GQ149730. ***S. betaceum*** Cav., Bolivia, *Bohs 2468* (UT); AF244713, AY996387, DQ180426. ***S. bolivianum*** Britton, Bolivia, *Nee 51814* (NY); GU591061, GU591113, GU591003. ***S. bonariense*** L., Argentina, *Barboza & al. 1567* (CORD); GU591062, GU591114, GU591004. ***S. buddleifolium*** Sendtn., Brazil, *Agra 7164* (JPB); GU591063, GU591115, GU591005. ***S. campechiense*** L., Costa Rica, *Bohs 2536* (UT); AF244728, AY996389, DQ180475. ***S. candidum*** Lindl., Costa Rica, *Bohs 2898* (UT); AF244722, AY562953, AY266237. ***S. caricaefolium*** Rusby, Bolivia, *Bohs 2741* (UT); GU591064, GU591116, GU591006. ***S. carolinense*** L., BIRM S.1816, *Olmstead S-77* (WTU); AY996491, AY996392, DQ180476. ***S.* cf. Mentz sp. 1**, Brazil, *Agra & al. 7274* (JPB); HQ457403, HQ457422, HQ457413. ***S. chrysotrichum*** Schltdl., Costa Rica, *Soto & al. 1222* (UT); GU591065, GU591117, GU591007. ***S. cinereum*** R. Br., NIJ 904750120, *Bohs 2852* (UT); AY996493, AY996394, DQ180397. ***S. citrullifolium*** A. Braun, Texas, *Urbatsch 4834* (NY); GQ143647, GQ143679, GQ149732. ***S. comarapanum*** M. Nee, Bolivia, *Nee 51861* (NY); GU591066, GU591118, GU591008. ***S. comptum*** C.V. Morton, Paraguay, *Bohs 3193* (UT); AY996498, AY996399, GU591009. ***S. cordovense*** Sessé & Moç, Costa Rica, *Bohs 2693* (UT); AF244717, AY996401, DQ180480. ***S. coriaceum*** Dunal, NIJ A44750296, *Stern 425* (UT); GU591067, GU591119, GU591010. ***S. crinitipes*** Dunal, Colombia, *Olmstead S-81* (WTU); AY996500, AY996402, DQ180481. ***S. crinitum*** Lam., Brazil, *Agra & al. 7028* (JPB); GQ143651, GQ143683, GQ149736. ***S. crotonoides*** Lam., Dominican Republic, *Nee 52298* (NY); AY996502, AY996404, GU591011. ***S. decompositiflorum*** Sendtn., Brazil, *Agra & al. 7206* (JPB); GU591068, GU591120, GU591012. ***S. decorum*** Sendtn., Brazil, *Agra & al. 7263* (JPB); HQ457396, HQ457415, HQ457405. ***S. diploconos*** (Mart.) Bohs, Brazil, *Bohs 2335* (UT); AY523890, AY996407, DQ180429. ***S. donianum*** Walp., Mexico, *Bohs 3472* (UT); GU591069, GU591121, GU591013. ***S. dulcamara*** L., Cult. Michigan USA, no voucher; AF244742, AY996410, AY226231. ***S. elaeagnifolium*** Cav., Paraguay, *Bohs 3204* (UT); AY996508, AY996412, DQ180399. Texas, *Olmstead S-82* (WTU); AF244730, AY996413, GU591014. ***S. ensifolium*** Dunal, Puerto Rico, *Bohs 2461* (UT); AY996506, AY996409, DQ180483. ***S. furfuraceum*** R. Br., BIRM S.1442, *Olmstead S-84* (WTU); AY996512, AY996417, DQ180401. ***S. gardneri*** Sendtn., Brazil, *Agra & al. 7195* (JPB); GU591070, GU591122, GU591015. ***S. glaucophyllum*** Desf., No voucher; AF244714, AY996418, DQ180430. ***S. glutinosum*** Dunal, NIJ A34750191, *Bohs 3262* (UT); AY996513, AY996419, GU591016. ***S. grayi*** Rose, Mexico, *Vallejo-Marin 07s195* (MEX); GQ143659, GQ143690, GQ149744. ***S. hasslerianum*** Chodat, Argentina, *Barboza & al. 2121* (CORD); GU591071, GU591123, GU591071. ***S. hexandrum*** Vell., Brazil, *Agra & al. 7104* (JPB); GU591072, GU591124, GU591018. ***S. hieronymi*** Kuntze, Argentina, *Nee & Bohs 50761* (NY); AY996517, AY996423, GU591019. ***S. hindsianum*** Benth., Mexico, *Bohs 2975* (UT); AY996518, AY996424, DQ180402. ***S. hirtum*** Vahl, Ecuador, *Whalen 730* (QCA); AY263462, AY996425, AY266254. ***S. hoehnei*** C.V. Morton, Brazil, *Folli 1668* (MO); AY996519, AY996426, DQ180484. ***S. jabrense*** Agra & M. Nee, Brazil, *Agra & al. 7016* (JPB);

Appendix. Continued.

GU591073, GU591125, GU591020. ***S. jamaicense*** Mill., BIRM S.1209, *Olmstead S-85* (WTU); AF244724, AY562956, DQ180485. ***S. juvenale*** Thell., Argentina, *Barboza & al. 2071* (CORD); GU591074, GU591126, GU591021. ***S. laciniatum*** Aiton, New Zealand, *Bohs 2528* (UT); AF244744, AY996431, DQ180467. ***S. lanceifolium*** Jacq., Mexico, *Aguilar & al. 1130* (MO); GU591075, GU591127, GU591022. ***S. lanceolatum*** Cav., Costa Rica, *Bohs 2728* (UT); AY996523, AY996432, GU591023. ***S. lasiocarpum*** Dunal, Thailand, *Heiser 8008* (IND); AY263457, –,–. *Ansyar 9605* (IND) –, AY996433, AY266256. ***S. leucopogon*** Huber, Peru, *Stern & al. 152* (UT); GU591076, GU591128, GU591024. ***S. lycocarpum*** A. St.-Hil., Paraguay, *Bohs 3212* (UT); AY996525, AY996435, DQ812107. ***S. megalonyx*** Sendtn., Brazil, *Agra & al. 7090* (JPB); GU591077, GU591129, GU591025. ***S. melongena*** L., BIRM S.0657, *Olmstead S-91* (WTU); GU591078, AY562959, DQ180406. ***S. metrobotryon*** Dunal, Brazil, *Agra & al. 7275* (JPB); HQ457397, HQ457416, HQ457406. ***S. microphyllum*** (Lam.) Dunal, Dominican Republic, *Nee 52300* (NY); AY996529, AY996441, GU591026. ***S. mitlense*** Dunal, Mexico, *Whalen & Velasco 825* (BH); AY996530, AY996442, DQ812108. ***S. monachophyllum*** Dunal, Guyana, *Stern 256* (UT); GU591079, GU591130, GU591027. ***S. mortonii*** Hunz., Argentina, *Barboza & al. 639* (CORD); GU591080, GU591131, GU591028. ***S. moxosense*** M. Nee, Bolivia, *McClelland & Stern 408* (NY); GU591081, GU591132, GU591029. ***S. multispinum*** N.E. Br., Paraguay, *Bohs 3198* (UT); AY996533, AY996444, GU591030. ***S. nemorense*** Dunal, Bolivia, *Bohs & Nee 2757* (UT); AY996536, AY996447, DQ180488. ***S. palinacanthum*** Dunal, Bolivia, *Bohs 3151* (UT); AY561268, AY562961, AY266233. ***S. paludosum*** Moric., Brazil, *Agra & al. 7003* (JPB); GU591082, GU591133, GU591031. ***S. paniculatum*** L., Paraguay, *Bohs 3181* (UT); AY996540, AY996452, GU591032. ***S. paraibanum*** Agra, Brazil, *Agra & al. 7008* (JPB); GU591083, GU591134, GU591033. ***S. pedemontanum*** M. Nee, Ecuador, *Bohs 3337* (UT); GU591084, GU591135, GU591034. ***S. piluliferum*** Dunal, Brazil, *Agra & al. 7295* (JPB); HQ457398, HQ457417, HQ457407. ***S. pluviale*** Standl., Costa Rica, *Soto & al. 1225* (UT); GU591085, GU591136, GU591035. ***S. poinsettiifolium*** Rusby, Bolivia, *McClelland & Stern 414* (NY); GU591086, GU591137, GU591036. ***S. polytrichum*** Moric., Brazil, *Agra & al. 7099* (JPB); GU591087, GU591138, GU591037. ***S. pseudocapsicum*** L., BIRM S.0870, no voucher; AF244720, AY562963, DQ180436. ***S. ptychanthum*** Dunal, Chicago, *Olmstead S-94* (WTU); AF244735, AY996457, DQ180454. **S.** ***reflexiflorum*** Moric. ex Dunal, Brazil, *Agra 7182* (JPB); GU591088, GU591139, GU591038. ***S. refractum*** Hook. & Arn., Mexico, *Iltis & al. 29694* (WIS); AY996547, AY996460, HQ457408. ***S. rhytidoandrum*** Sendtn., Argentina, *Nee & Bohs 50861* (NY); GU591089, GU591140, GU591039. ***S. robustum*** H.L. Wendl., Argentina, *Bohs 3084* (UT); AY561270, AY562966, AY266259. ***S. rostratum*** Dunal, NIJ 934750126, *Cipollini 173* (UT); GQ143670, GQ143702, GQ149755. ***S. rudepannum*** Dunal, Costa Rica, *Soto & al. 1223* (UT); GU591090, GU591141, GU591040. ***S. rupincola*** Sendtn., Brazil, *Thomas & al. 1571* (MO); GU591091, GU591142, GU591041. ***S. schomburghii*** Sendtn., Suriname, *Clarke 11269* (US); GU591092, GU591143, GU591042. ***S. scuticum*** M. Nee, Brazil, *Agra & al. 7242* (JPB); HQ457400, HQ457419, HQ457410. ***S. sendtnerianum*** Van Huerck & Müll. Arg., Brazil, *da Cunha & Wang 310* (MO); GQ143671, GQ143703, GQ149756. ***S. sisymbriifolium*** Lam., Argentina, *Bohs 2533* (UT); AY561271, AY562967, AY266235. ***Solanum* sp. nov. Brazil,** Brazil, *Agra & al. 7108* (JPB); HQ457399, HQ457418, HQ457409. ***S. stagnale*** Moric., Brazil, *Bohs 3094* (UT); GU591093, GU591144, GU591043. ***S. stellativelutinum*** Bitter, Bolivia, *Wood & al. 18688* (NY); GU591094, GU591145, GU591044. ***S. stenandrum*** Sendtn., Brazil, *Irwin & al. 33085* (WIS); AY561273, AY562969, AY559242. ***S. stramoniifolium*** Jacq., Peru, *Whalen 860* (HUT); AY263465, AY562970, AY266263. ***S. subinerme*** Jacq., Panama, *Ebinger 321* (US); GU591095, GU591146, GU591045. ***S. subumbellatum*** Vell., Brazil, *Agra & al.* 7260 (JPB); HQ457401, HQ457420, HQ457411. S. ***talarense*** Svenson, Peru, *Sagastegui & Leiva 15487* (NY); GU591096, GU591147, GU591046. ***S. tampicense*** Dunal, U.S.A., no voucher; GU591097, GU591148, GU591047. ***S. tetramerum*** Dunal, Dominican Republic, *Garcia & al. 5909* (MO); GU591098, GU591149, GU591048. ***S. thomasiifolium*** Sendtn., Brazil, *Tavares & al. 5909* (MO); GU591099, GU591150, GU591049. ***S. torvum*** Sw., BIRM S.0839, *Olmstead S-101* (WTU); GU591100, AY562972, AY266246. ***S. tridynamum*** Dunal, BIRM S.1831, *Olmstead S-102* (WTU); GU591101, AY996474, DQ180412. ***S. ursinum*** Rusby, Bolivia, *Nee 51767* (NY); GU591102, GU591151, GU591050. ***S. urticans*** Dunal, Bolivia, *Bohs 2759* (UT); GU591103, GU591152, GU591051. ***S. vaillantii*** Dunal, Brazil, *Agra & al. 7238* (JPB); HQ457402, HQ457421, HQ457412. ***S. viarum*** Dunal, NIJ 934750190, *Cipollini 67* (UT); AY561275, AY562973, AY559243. ***S. wendlandii*** Hook.f., BIRM S.0488, no voucher; AF244731, AY562974, DQ180440. ***S. whalenii*** M. Nee, Bolivia, *Nee 51765* (UT); GU591104, GU591153, GU591052. ***S. wrightii*** Benth., Costa Rica, *Bohs 2445* (UT); GQ480731, GQ480733, GQ480732.