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Phylogeny of the Carolinense Clade of *Solanum* (Solanaceae) Inferred from Nuclear and Plastid DNA Sequences

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Abstract—The large and economically important genus *Solanum* contains ca. 1,400 species distributed worldwide. One of the 12–14 major clades identified in the genus is the Leptostemonum clade, or the "spiny solanums." Previous molecular phylogenetic studies have identified 14 major clades in the spiny solanums. Among these is the Carolinense clade, which contains *Solanum carolinense*, the type species of *S.* subsect. *Lathyrocarpum*. However, previous results have suggested that the species composition of the Carolinense clade is only partially congruent with taxonomic circumscriptions of *S.* subsect. *Lathyrocarpum*. The main goal of this study was to increase taxon sampling over previous molecular phylogenies in order to better understand the composition of the Carolinense clade. We newly sequenced 31 accessions of 17 taxa putatively belonging to the clade for two nuclear (ITS, *waxy*) and one plastid gene region (*trnT–trnF*) and combined these with previously generated molecular data. Phylogenetic analyses of separate and combined DNA matrices were done using maximum parsimony and Bayesian methods. Results from analyses of the combined nuclear and plastid data set showed 10 taxa to be resolved within a well-supported Carolinense clade. However, three species, *Solanum dimidiatum*, *S. perplexum*, and *S. hieronymi*, showed conflicting placements in phylogenies based on analyses of nuclear vs. plastid data sets, suggesting a history of allopolyploidy or introgression. Within the Carolinense clade, the North and South American species were both recovered as well-supported clades, implying a single dispersal event from South to North America.

Keywords—Allopolyploidy, granule-bound starch synthase (GBSSI), hybridization, phylogeny, *Solanum* subsect. *Lathyrocarpum*, *Solanum carolinense*.

The genus *Solanum* L. (Solanaceae), with a worldwide distribution and ca. 1,400 species, is among the largest genera of flowering plants and contains several economically important species (Bohs 2005). Previous molecular phylogenetic studies in *Solanum* have identified 12–14 major clades in the genus (Bohs 2005; Weese and Bohs 2007), including the large Leptostemonum clade, which corresponds closely to *Solanum* subg. *Leptostemonum* (Dunal) Bitter. *Solanum* subg. *Leptostemonum*, often referred to as the "spiny solanums," consists of 350–450 species distributed worldwide and includes the cultivated eggplant, *S. melongena* L. Species placed in the subgenus typically share the unique combination of stellate hairs, prickles, and tapered anthers (Whalen 1984).

Whalen (1984) and Nee (1999) provided synopses of infrageneric groupings in *Solanum* subg. *Leptostemonum* based on morphological characters. Whalen (1984) proposed 33 informal species groups within the subgenus and left 36 species unplaced. He included seven South American species in the *Solanum multispinum* species group (Table 1), characterized by erect or prostrate rhizomatous plants with abundant needle-like prickles, usually densely spiny calyces, white to violet corollas, a weakly andromonoecious reproductive system, and glabrous, small- to medium-sized green to yellow fruits. Among the North American spiny solanums, he left *S. carolinense* (the type species of *S.* subsect. *Lathyrocarpum* G. Don) and *S. dimidiatum* unplaced, although they both exhibit most of the morphological characters used to define the *S. multispinum* species group. *Solanum pumilum* was not included in his classification, and two other taxa were treated as synonyms: *S. carolinense* var. *floridanum* under var. *carolinense*, and *S. perplexum* under *S. dimidiatum* (Whalen 1984).

Nee (1999) treated the New World species of spiny solanums, placing them into 10 sections. In *Solanum* sect. *Melongena* (Mill.) Dunal subsect. *Lathyrocarpum*, Nee (1999) included 17 described and two undescribed species that he distributed into four series [see Table 1; all three species of Nee's series 4 were found to belong to the Elaeagnifolium clade (Levin et al. 2006; Stern et al. 2011) and are not listed]. Series 1 through 3 of Nee's *S.* subsect. *Lathyrocarpum* included all the taxa of Whalen's *S. multispinum* group, three species not treated by Whalen (*S. mortonii*, *S. pumilum*, and *S. moxosense*), four species Whalen left unplaced (*S. carolinense*, *S. chamaeacanthum*, *S. dimidiatum*, *S. euacanthum*), and *S. hasslerianum*, which was placed in Whalen's *S. polytrichum* species group.

Recent phylogenetic studies have aimed to identify and to delimit monophyletic groups within *Solanum* subg. *Leptostemonum* and to compare them with the species groups and infrageneric taxa proposed by Whalen (1984) and Nee (1999), respectively. Levin et al. (2006) provided the first large-scale molecular phylogeny of the spiny solanums based on data from three molecular markers. They identified 10 major well-supported clades within the spiny solanums, one of which was the Carolinense clade. In their sampling scheme, the clade consisted of the North American *S. carolinense* and two South American species, *S. comptum* and *S. conditum* (the latter now known as *S. aridum*). The Carolinense clade was also recovered in the expanded phylogeny of spiny solanums by Stern et al. (2011) using slightly different molecular markers and additional taxon sampling. In their study, the Carolinense clade was composed of *S. carolinense* and four South American species: *S. aridum*, *S. comptum*, *S. juvenale*, and *S. moxosense*. Levin et al. (2006) and Stern et al. (2011) also sampled single accessions of *S. hieronymi* and *S. multispinum* from Whalen's *S. multispinum* group, but in both molecular studies these species fell outside the Carolinense clade. Both studies also recovered the three species of Nee's series 4 (*S. elaeagnifolium*, *S. hindsianum*, *S. tridynamum*) in a strongly supported Elaeagnifolium clade.

Throughout this paper, the infrageneric taxon *Solanum* subsect. *Lathyrocarpum* is used in reference to the most recent

TABLE 1. A list of taxa comparing their placement in the taxonomic treatments of Whalen (1984) and Nee (1999) and the molecular clade in which each was recovered in the phylogenies of Levin et al. (2006), Stern et al. (2011), and this study. All three phylogenies are based on the analysis of combined nuclear and plastid data sets. A dash (—) indicates a taxon which was not treated in Whalen (1984) or Nee (1999) or was not included in a phylogeny.

	Taxonomic concepts		Molecular clades		
Taxon	Whalen (1984) Informal species group	Nee (1999) Series of *S.* subsect. *Lathyrocarpum*	Levin et al. 2006	Stern et al. 2011	Wahlert et al. (this study)
S. carolinense L. var. *carolinense*	Incertae sedis	Series 1	Carolinense	Carolinense	Carolinense
S. carolinense var. *floridanum* Chapm.	Incertae sedis (as a synonym of *S. carolinense*)	—	—	—	Carolinense
S. chamaeacanthum Griseb.	Incertae sedis	Series 3	—	—	—
S. comptum C. V. Morton	S. *multispinum* group	Series 2	Carolinense	Carolinense	Carolinense
S. conditum C. V. Morton (= *S. aridum* Morong)	S. *multispinum* group	Series 2	Carolinense	Carolinense	Carolinense
S. dimidiatum Raf.	Incertae sedis	Series 1	—	—	Carolinense
S. euacanthum Phil.	Incertae sedis	Series 2	—	—	Unplaced
S. flagellare Sendtn.	S. *multispinum* group	Series 3	—	—	—
S. hasslerianum Chodat	S. *polytrichum* group	Series 2	—	Sisymbriifolium	Sisymbriifolium
S. hieronymi Kuntze	S. *multispinum* group	Series 2	Unplaced	Unplaced	Unplaced
S. homalospermum Chiarini	—	—	—	—	Elaeagnifolium
S. juvenale Thell.	S. *multispinum* group	Series 2	—	Carolinense	Carolinense
S. mortonii Hunz.	—	Series 2	—	Elaeagnifolium	Elaeagnifolium
S. moxosense M. Nee (as *S.* sp. nov. ined, *M. Nee 34261*)	—	Series 3	—	Carolinense	Carolinense
S. multispinum N. E. Br.	S. *multispinum* group	Series 2	Unplaced	Unplaced	Unplaced
S. perplexum Small	Incertae sedis (as a synonym of *S. dimidiatum*)	—	—	—	Carolinense
S. pumilum Dunal	—	Series 2	—	—	Carolinense
S. reineckii Briq.	S. *multispinum* group	Series 3	—	—	Carolinense
S. savannarum Ribeiro-Silva & Proença (as *S.* sp. nov. ined, *Irwin & Soderstrom 7565*)	—	Series 2	—	—	—

circumscription by Nee (1999), and the name 'Carolinense clade' refers to the monophyletic group recovered in phylogenetic studies. Table 1 compares the species included in Whalen's *S. multispinum* group and Nee's circumscription of *S.* subsect. *Lathyrocarpum* with their phylogenetic placement in the molecular clades of Levin et al. (2006), Stern et al. (2011), and the present study.

In this study, we sequenced seven taxa in addition to those of Levin et al. (2006) and Stern et al. (2011), as well as 16 new accessions of previously sampled taxa. We sampled all species in Whalen's (1984) *Solanum multispinum* group and in Nee's (1999) series 1 through 3 of *S.* subsect. *Lathyrocarpum* except for *S. chamaeacanthum* and *S. flagellare*, for which we could not obtain leaf tissue. We included the recently described *S. homalospermum* (Chiarini 2004), a species thought to belong to the *Solanum multispinum* group but not included in previous phylogenetic studies. We also sampled *S. euacanthum*, a species that was placed in series 2 of *S.* subsect. *Lathyrocarpum* by Nee (1999), but also not included in any molecular phylogeny. The main goal of this study was to clarify the species composition of the Carolinense clade so that a taxonomic revision of the included species can proceed, a necessary strategy for a giant genus such as *Solanum*.

MATERIALS AND METHODS

Taxon Sampling—The taxon sampling strategy and outgroups used here were based on previous molecular phylogenetic studies in *Solanum* subg. *Leptostemonum* (Levin et al. 2006; Stern et al. 2011) and the taxonomic classifications of Whalen (1984) and Nee (1999) (see Table 1). We sampled seven taxa beyond those in Levin et al. (2006) and Stern et al. (2011) (*S. carolinense* var. *floridanum*, *S. dimidiatum*, *S. euacanthum*, *S. homalospermum*, *S. perplexum*, *S. pumilum*, and *S. reineckii*), as well as multiple accessions of taxa previously represented by a single exemplar (*S. aridum*, *S. carolinense* var. *carolinense*, *S. comptum*, *S. dimidiatum*, *S. hasslerianum*, *S. hieronymi*, *S. moxosense*, *S. multispinum*, *S. perplexum*, *S. pumilum*, *S. reineckii*, and *S. sisymbriifolium*). Newly sequenced taxa and accessions in this study are represented in bold font in the phylogenetic trees in Figs. 1–2. Voucher information and GenBank accession numbers are given in the Appendix.

DNA Extraction, Amplification, and Sequencing—Genomic DNA was isolated from herbarium specimens or fresh, silica-dried leaf tissue using the DNEasy plant mini kit (Qiagen, Valencia, California). The ITS 1, 5.8S, and ITS 2 regions of the internal transcribed spacer (ITS) were amplified as a whole using the primer pair ITS 4 + Leu 1, or in two fragments using the primer combinations ITS 3 + ITS 4 and Leu 1 + ITS 2C (White et al. 1990; Bohs and Olmstead 2001). The *trnT* (UGU)–*trnF* (GAA) region was amplified in either two fragments using the primer pairs Tab A + Tab D and Tab C + Tab F, or in three fragments using Tab A + Tab B, Tab C + Tab D, and Tab E + Tab F (Taberlet et al. 1991). The nuclear region containing exons 2 through 10 of the GBSSI gene (granule-bound starch synthase, or *waxy*) was amplified either in two parts using primer pairs *waxy*F + 1171R and 1058F + 2R, or in four parts using primer pairs *waxy*F + Ex4R, Ex4F + 1171R, 1058F + 3′N, and 3F + 2R (Levin et al. 2005). Each 25 µl reaction contained 1–10 ng DNA, 1.0 unit AmpliTaq gold polymerase (Applied Biosystems, Foster City, California), 2.5 µl 10 × buffer, 1.5 µl 20 mM $MgCl_2$, 1.0 µl 10 mM dNTPs, and 0.75 µl 2 mM amplification primer. For amplification of the ITS region, 1.0 µl each of DMSO and glycerol were added to the reaction. Conditions for PCR followed Levin et al. (2006) for the ITS region, Bohs and Olmstead (2001) for the *trnT–trnF* region, and Levin et al. (2005) for the *waxy* region. The PCR amplicons were visualized by electrophoresis on a 1.3% agarose gel stained with ethidium bromide and cleaned with the Promega Wizard® PCR clean-up system (Promega Corp., Madison, Wisconsin). Cycle-sequencing reactions used the same primers as in PCR and were visualized on an Applied Biosystems 3730 DNA Analyzer at the University of Utah HSC Core Research Facility, Salt Lake City, Utah. Sequence trace files were edited using Sequencher v4.8 (Gene Codes Corp., Ann Arbor, Michigan).

Four *trnS–trnG* sequences were generated for *S. perplexum* and three accessions of *S. dimidiatum* following Levin et al. (2006) and were combined in a four-gene (*trnS–trnG*, *trnT–trnF*, ITS, *waxy*), 59-taxon data set using data from Levin et al. (2006) and Stern et al. (2011). The matrix included four other species from the Carolinense clade (*S. aridum*, *S. carolinense*, *S. comptum*, and *S. hieronymi*) and included most of the same taxa as Levin et al. (2006). The resulting matrix was used to perform separate phylogenetic analyses of plastid sequences (*trnS–trnG* and *trnT–trnF*), nuclear sequences (ITS and *waxy*), and a combined data set.

Alignment and Phylogenetic Analyses—Newly generated sequences were added to the data matrix of Stern et al. (2011) using Se-Al v2.0a11 (Rambaut 1996), keeping two to six exemplars of previously identified clades of *Solanum* subg. *Leptostemonum* and outgroups. The matrix was then initially re-aligned using the MAFFT algorithm (Katoh et al. 2002) as implemented in the CIPRES Science Gateway v3.3 (Miller et al. 2010). Manual adjustments to the MAFFT alignment were made using BioEdit (Hall 2007). A 79-bp portion of the ITS region could not be unambiguously aligned (positions 93–171) and was excluded from all subsequent analyses. Gaps in the alignment arising from insertions and deletions were scored as missing data, and the aligned combined DNA matrix was deposited in TreeBASE (Study ID S14032). The plastid (*trnT–trnF*) and nuclear data sets (ITS and *waxy*) were analyzed separately and as a combined data set.

Maximum parsimony (MP) analyses were conducted on the three data matrices in PAUP* v4.0b10 (Swofford 2002) using a heuristic search strategy with TBR branch swapping, 1,000 random addition replicates, saving one tree per replicate, steepest descent off, and MULTREES in effect. All characters were equally weighted and unordered. Internal branch support values were estimated with 1,000 bootstrap (BS) replicates (Felsenstein 1985) using a full heuristic search with TBR branch swapping, 100 random stepwise addition replicates, and MULTREES in effect. Prior to conducting Bayesian analyses, the GTR + I + G model was chosen as the optimal of model nucleotide substitution for each data partition using the Akaike information criterion as implemented in MrModeltest v2 (Nylander 2004). Bayesian inference (BI) was conducted on the three different matrices using the CIPRES Science Gateway v3.3 (Miller et al. 2010). Two separate analyses (nruns = 2) were run for five million generations to estimate probabilities using the Markov chain Monte Carlo algorithm as implemented in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Trees were saved every 1,000th generation, with the first 25% of trees removed as "burn-in" and summarized in a 50% majority rule consensus tree where the proportion of clades on the tree represent BI posterior probabilities. All trees were rooted using *Solanum laciniatum* as the outgroup based on results given in Levin et al. (2006).

RESULTS

The combined DNA matrix of all three gene regions (ITS, *waxy*, and *trnT–trnF*) was 4,107 bp long and had 2.2% missing data. Summary statistics of each data set (ITS, *waxy*, and *trnT–trnF*) and the combined data set are given in Table 2.

For the purposes of this study, the results and discussion will be restricted primarily to the taxa of interest listed in Table 1. The MP and BI analyses of the combined data matrix and the nuclear data set (ITS and *waxy*) resulted in largely congruent trees, but the plastid *trnT–trnF* data set resulted in a different phylogenetic placement for three species (see below). The main difference between the MP and BI analyses was that BI resulted in more highly resolved trees. Tree topologies from Bayesian analyses were not in conflict with those of MP strict consensus trees when weakly supported nodes (i.e. BI posterior probability ≤ 0.90) were collapsed to polytomies. Therefore, results of phylogenetic analyses are presented as more conservative MP strict consensus cladograms with branch support values indicated by both MP bootstrap percentages and BI posterior probabilities (Figs. 1–2; support values are reported throughout as MP bootstrap percentage/BI posterior probability).

Maximum parsimony analysis of the combined data matrix (ITS, *waxy*, and *trnT–trnF*) yielded 7,819 most parsimonious trees that are summarized in a strict consensus tree in Fig. 1. The overall topology was similar to that in Stern et al. (2011); the Leptostemonum clade was unambiguously supported (100/1.0) and the same 14 major clades were recovered with comparable branch support. Among the taxa of interest, 10 were placed in the Carolinense clade (72/0.91), including five taxa not sampled in previous studies (*S. carolinense* var. *floridanum*, *S. dimidiatum*, *S. pumilum*, *S. perplexum*, and *S. reineckii*). *Solanum carolinense*, *S. comptum*, *S. moxosense*, *S. pumilum*, and *S. reineckii* were represented by more than one accession and were strongly supported (100/1.0) as monophyletic. *Solanum aridum* and *S. perplexum* were each represented by three accessions and were recovered as weakly supported clades (70/0.80 and 70/0.94, respectively). *Solanum carolinense* var. *carolinense*, represented by four accessions, was resolved as paraphyletic with respect to var. *floridanum*, but relationships within this group were poorly resolved. Accessions 1, 2, and 3 of *S. dimidiatum* were recovered in a strongly supported clade (97/1.0), but the species was non-monophyletic because accession 4 was sister to the North American taxa (Fig. 1). Within the Carolinense clade, two subclades were recovered that corresponded to the taxa from North America (*S. carolinense* var. *carolinense*, *S. carolinense* var. *floridanum*, *S. dimidiatum*, *S. perplexum*, and *S. pumilum*; 87/1.0) and South America (*S. aridum*, *S. comptum*, *S. juvenale*, *S. moxosense*, and *S. reineckii*; 92/1.0).

The analyses of the combined data set recovered the three accessions of *Solanum hieronymi* in a strongly supported clade (100/1.0) which was placed within a larger clade containing the Elaeagnifolium and Old World clades (94/1.0; Fig. 1). *Solanum euacanthum* was weakly supported as sister to the Elaeagnifolium clade (65/< 0.50), and *S. homalospermum* was nested within the Elaeagnifolium clade. Three accessions of *S. multispinum* formed a strongly supported clade (100/1.0), but its relationship to other clades was unresolved. Both *S. hasslerianum* and *S. sisymbriifolium* were recovered in a strongly supported Sisymbriifolium clade (100/1.0). Although neither species was resolved as monophyletic, relationships within this clade were poorly resolved and poorly supported.

While the separate analyses of ITS and *waxy* data sets resulted in trees with a similar topology to each other and to the combined data set, the analysis of the *trnT–trnF* data set showed a different pattern of relationships among the taxa of the Carolinense clade. Abbreviated trees showing the taxa of

TABLE 2. Descriptive statistics for each data partition, the ITS and *waxy* combined matrix, and the combined matrix of all three gene regions (ITS, *waxy*, and *trnT–trnF*). PI, parsimony informative; MPTs, most parsimonious trees; CI, consistency index; RI, retention index.

Data partition	Aligned sequence length	Constant characters (%)	PI characters	Number of MPTs	Tree length (steps)	CI	RI
ITS	599	280 (46.7%)	180 (30.1%)	15	1,152	0.415	0.690
waxy	1,718	993 (57.8%)	454 (26.4%)	$> 1 \times 10^5$	1,314	0.711	0.844
ITS + *waxy*	2,317	1,273 (54.9%)	634 (27.4%)	2,648	2,558	0.552	0.751
trnT–trnF	1,790	1,488 (80.9%)	183 (10.2%)	$> 1 \times 10^6$	503	0.793	0.881
Combined	4,107	2,721 (66.3%)	819 (19.9%)	7,819	3,140	0.580	0.761

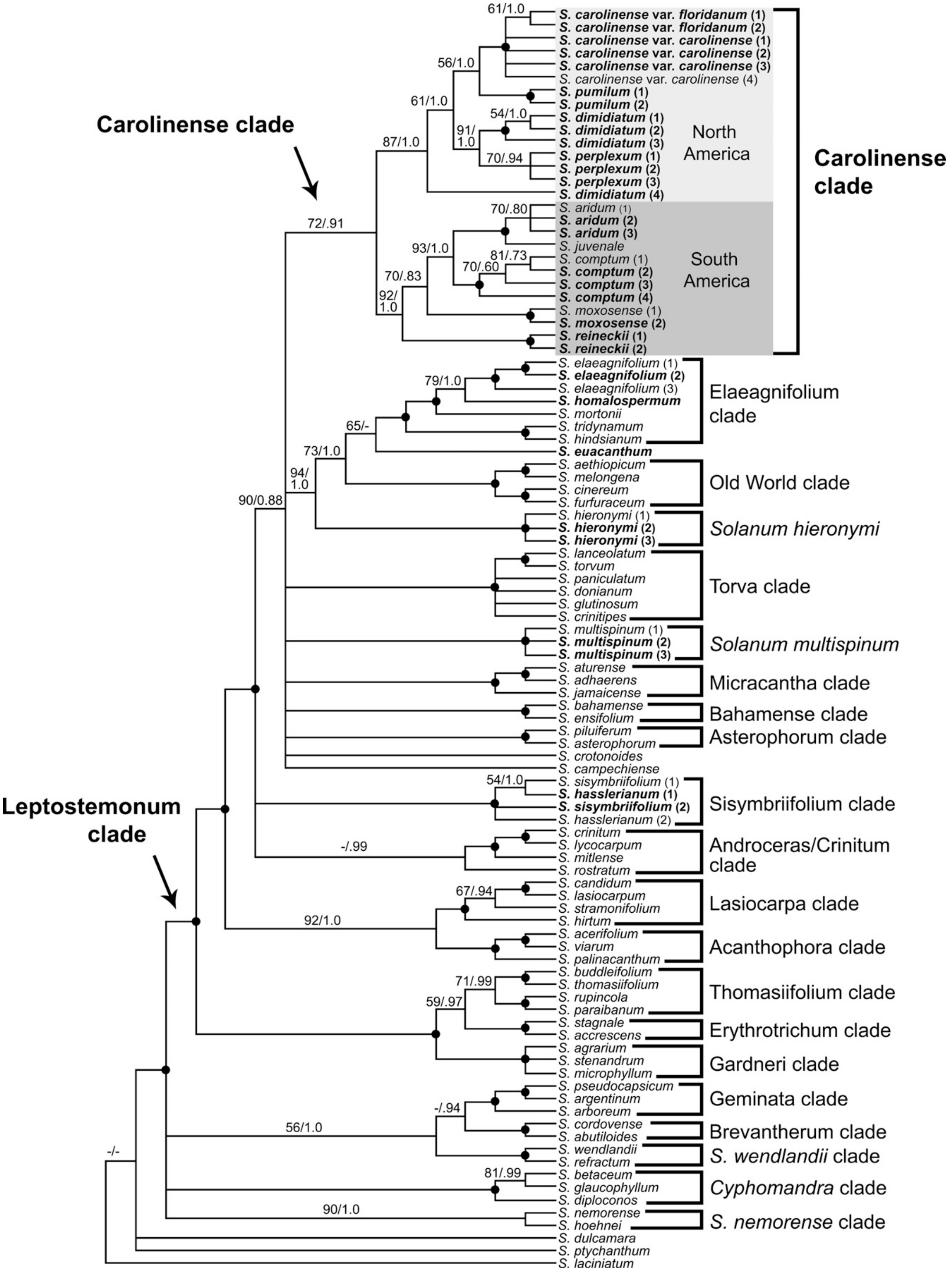
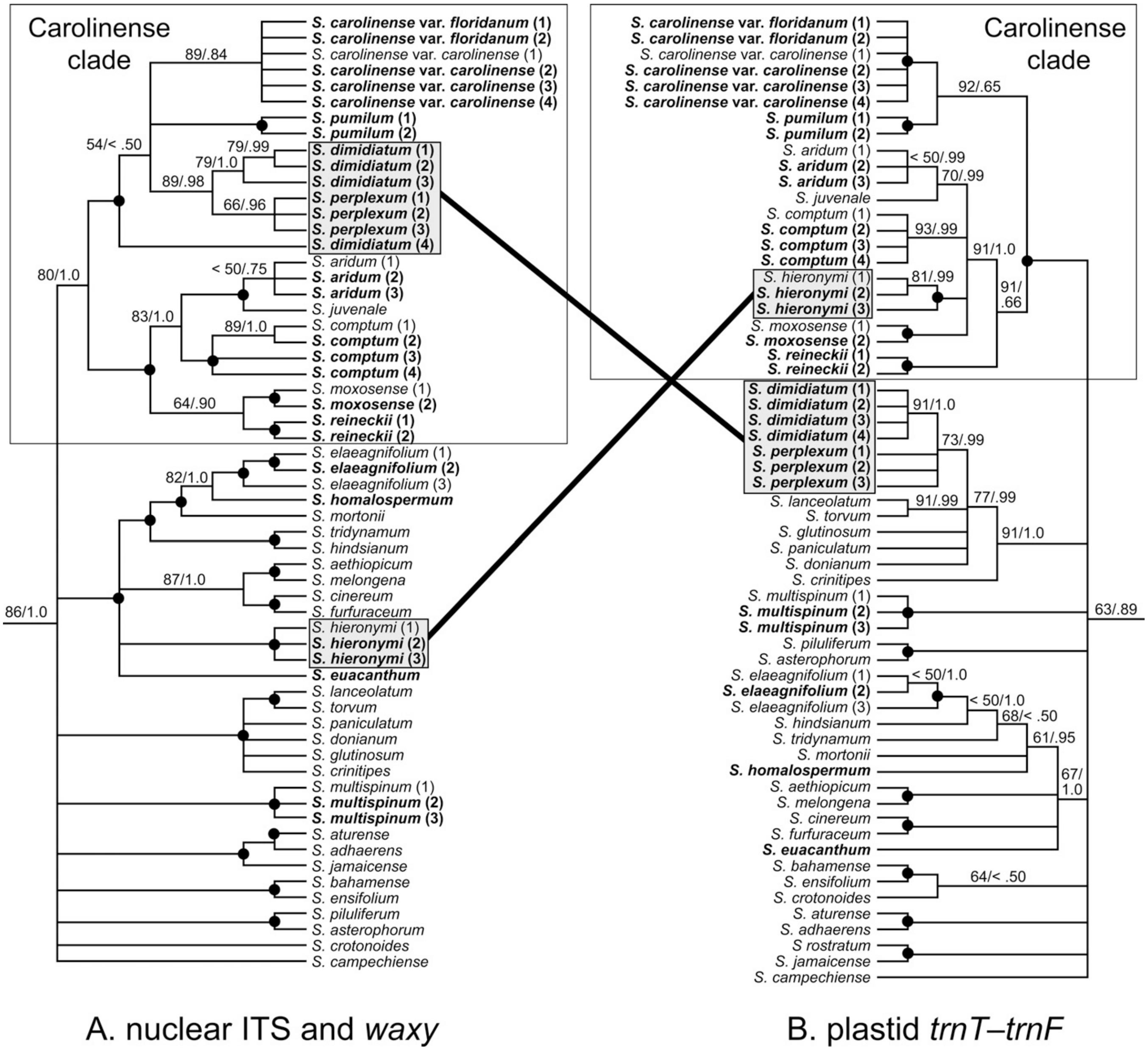


FIG. 1. Strict consensus tree derived from MP analysis of the combined data set (ITS, *waxy*, and *trnT–trnF*). Values above the branches are MP bootstrap percentages (> 50%) followed by posterior probabilities calculated from Bayesian inference (BI); values less than 50% bootstrap or 0.50 probability are represented by a dash (-). Nodes supported by a combined value of ≥ 95% MP bootstrap and ≥ 0.95 BI posterior probability are represented by a solid black circle. Taxa in bold are those sampled in addition to Stern et al. 2011. Clade names follow Stern et al. 2011.



A. nuclear ITS and *waxy*

B. plastid *trnT–trnF*

FIG. 2. Strict consensus trees derived from MP analysis of the nuclear data set (A) and the plastid (B) data set showing different relationships of *S. dimidiatum*, *S. perplexum*, and *S. hieronymi*. Trees are abbreviated showing only the taxa and clades of interest (see Table 1). Values above the branches are MP bootstrap percentages (> 50%) followed by posterior probabilities calculated from Bayesian inference (BI). Nodes supported by a combined value of ≥ 95% MP bootstrap and ≥ 0.95 BI posterior probability are represented by a solid black circle. Taxa in bold are those sampled in addition to Stern et al. 2011.

interest in Fig. 2 reveal two different topologies recovered from analyses of the nuclear (ITS and *waxy*) data set (Fig. 2a) and the plastid (*trnT–trnF*) data set (Fig. 2b). Analyses of the nuclear data set recovered the Carolinense clade (80/1.0) with the same species composition as the combined analysis (Figs. 1, 2a), with *S. perplexum* in a weakly supported clade (66/0.96) and accessions 1, 2, and 3 of *S. dimidiatum* in a moderately supported clade (79/1.0). *Solanum hieronymi* was resolved within a strongly supported clade (100/1.0) which also contained the Elaeagnifolium and Old World clades + *S. euacanthum* (Fig. 2a), comparable to its position in the combined analysis.

Analyses of the *trnT–trnF* data set showed a different pattern of relationships. The seven accessions of *S. dimidiatum* and *S. perplexum* were resolved in a weakly supported clade (73/0.99) and were part of a larger clade containing six species of the Torva clade (91/1.0; Fig. 2b). The three accessions of *Solanum hieronymi* were resolved as a strongly supported monophyletic group (100/1.0) nested among the South American taxa in a strongly supported Carolinense clade (99/1.0; Fig. 2b). Finally, in a separate analysis of the combined nuclear and plastid data sets, which excluded *S. dimidiatum*, *S. perplexum*, and *S. hieronymi*, node support for the Carolinense clade and the North American subclade each increased to 100/1.0, whereas support for the South American subclade decreased slightly to 89/1.0 (tree not shown).

The plastid *trnS–trnG* data set of 59 taxa, which included eight exemplars from the Carolinense clade, was analyzed as a single data set and in combination with the other data sets (*trnT–trnF*, ITS, *waxy*). When the *trnS–trnG* data set was analyzed separately, *Solanum hieronymi* was recovered in a clade with two other South American species of the Carolinense clade (*S. aridum* and *S. comptum*), a result similar to that from the *trnT–trnF* data set (Fig. 2b). The positions of *S. carolinense*, *S. dimidiatum*, and *S. perplexum* were unresolved in the *trnS–trnG* tree due to low sequence variability and few parsimony informative positions. When the *trnS–trnG* data set was combined and analyzed with the *trnT–trnF* data set, a similar topology was recovered as in the separate *trnT–trnF* analysis (Fig. 2b). *Solanum dimidiatum* and *S. perplexum* were recovered within a clade containing species from the Torva clade (76% MP bootstrap), and *S. carolinense* and *S. hieronymi* were placed in a clade containing two other species from the Carolinense clade (82% MP bootstrap). When all four data sets (*trnS–trnG*, *trnT–trnF*, ITS, *waxy*) were combined and analyzed, the resulting topology was the same as that recovered from the three-gene analysis shown in Fig. 1.

Discussion

The increased taxon sampling in this study has led to a better understanding of the taxa belonging to the Carolinense clade. The Carolinense clade recovered in the analyses of nuclear and combined data sets corresponded only in part to the species included in the *Solanum multispinum* species group by Whalen (1984) and *S.* subsect. *Lathyrocarpum* by Nee (1999). Results presented here have expanded on the findings of Levin et al. (2006) and Stern et al. (2011) and have shown the clade to be composed of certain taxa from Nee's series 1–3 of *S.* subsect. *Lathyrocarpum* (Nee 1999) and most but not all of the taxa of the *S. multispinum* species group of Whalen (1984). Four species (*S. euacanthum*, *S. hasslerianum*, *S. mortonii*, and *S. multispinum*) included in *S.* subsect. *Lathyrocarpum* by Nee (1999) are not resolved within the Carolinense clade. *Solanum homalospermum*, not treated by Whalen (1984) or Nee (1999), but postulated by Chiarini (2004) to belong to the *S. multispinum* group, is also excluded from the clade.

Relationships within the Carolinense Clade—Regardless of the analyses of the separate or combined data sets, eight taxa were consistently placed in the Carolinense clade: *S. aridum*, *S. carolinense* var. *carolinense*, *S. carolinense* var. *floridanum*, *S. comptum*, *S. juvenale*, *S. moxosense*, *S. pumilum*, and *S. reineckii*. Three species, *S. dimidiatum*, *S. perplexum*, and *S. hieronymi*, were either recovered in the Carolinense clade or not, depending on the analysis of nuclear vs. plastid DNA sequences (see below). When the three species were removed from the combined analysis, node support for the Carolinense clade was unambiguous (100/1.0).

Among the taxa of the Carolinense clade, five have a native distribution in North America (*S. carolinense* var. *carolinense*, *S. carolinense* var. *floridanum*, *S. dimidiatum*, *S. perplexum*, and *S. pumilum*) and the rest are native to South America. Analysis of the combined data set recovered the North American species in a moderately supported clade (87/1.0) and the South American species in a well-supported clade (92/1.0; Fig. 1). Given that the North American species are nested among a large clade of mainly Neotropical species in the overall phylogeny, it is reasonable to hypothesize a single dispersal to North America and subsequent diversification from a South American progenitor.

Solanum carolinense var. *carolinense* has a large native range in the eastern half of the U. S. A. and parts of southernmost Canada, and has become a problematic invasive weed in many areas around the world (e.g. Wehtje et al. 1987; Follak and Strauss 2010). With the exception of the narrowly endemic *S. pumilum*, which is currently only known from a few dolomite glades in Alabama, the other North American species of the Carolinense clade are weedy and grow in disturbed areas such as roadsides, cultivated fields, pastures, and urban waste areas. *Solanum carolinense* var. *floridanum*, which is sometimes treated as a synonym of var. *carolinense*, is distinguished morphologically by its smaller leaves with rounded lobes and deep sinuses that reach almost to the midrib. When the two varieties are found in proximity to one another at a local scale, var. *floridanum* usually occurs in more mesic sites (L. C. Anderson, pers. comm.). In the combined DNA data set, the two accessions of var. *floridanum* had a single synapomorphic nucleotide substitution in the ITS region. When compared to var. *carolinense*, the mean uncorrected p-distance between the two varieties was 0.42%, which was less than the 0.57% divergence among the four accessions of var. *carolinense*. Notwithstanding the fact that there is a greater sequence divergence among accessions of var. *carolinense* than between the two varieties, there remains adequate morphological and ecological differentiation to recognize var. *floridanum* as distinct from var. *carolinense*.

Since its description in 1933, *Solanum perplexum* from Alabama, Florida, and Georgia, U. S. A. has been placed in synonymy by most authors under *S. dimidiatum*, which is disjunctly distributed in Texas, Oklahoma, Louisiana, Arkansas, and Kansas. The mean uncorrected p-distance among the three accessions of *S. perplexum* was 0.57%, and among accessions 1, 2, and 3 of *S. dimidiatum* was 0.27%. The mean p-distance between the two species was 0.61%. Even with the relatively small divergence of DNA sequences between the species, the non-overlapping distributions and several morphological differences between *S. perplexum* and *S. dimidiatum* argue for the reinstatement of the former as a separate species. Results from the combined and nuclear analyses placed accession 4 of *S. dimidiatum* as sister to the North American taxa (87/1.0) and rendered the species non-monophyletic. Accession 4 is morphologically atypical in that it lacks prickles and has entire leaves, unlike the moderately to densely spiny plants with deeply lobed leaves that are characteristic of *S. dimidiatum*. It is not known if accession 4 represents a new species or if it is from a localized population of hybrid origin, which would explain its phylogenetic placement and its divergent morphology.

Among the South American members of the Carolinense clade (including *Solanum hieronymi*), four species (*S. aridum*, *S. comptum*, *S. hieronymi*, and *S. juvenale*) occur primarily in Argentina but also range into Bolivia and Paraguay. Chiarini (2007) has identified these four species as comprising two taxonomically similar species pairs: *S. aridum*/*S. juvenale* and *S. comptum*/*S. hieronymi*. In the analysis of the combined matrix, *Solanum juvenale* and the three accessions of *Solanum aridum* were recovered in a highly supported clade (100/1.0; Fig. 1). *Solanum aridum* and *S. juvenale* are similar morphologically, but are distinguished by leaf size and the density and length of prickles. They also differ in their ploidy levels, with *S. aridum* being a diploid ($n = 12$) and *S. juvenale* a tetraploid ($n = 24$; Moscone 1992; Chiarini 2007). Chiarini (2007) reports that experimental crosses between the two species result in triploids ($n = 18$) with reduced fertility, and that the two may naturally hybridize in mixed populations in northern Argentina. *Solanum comptum* and *S. hieronymi* form another species pair, but *S. comptum* is readily differentiated from *S. hieronymi* by its smaller globose fruits and strongly accrescent calyces. They also differ in chromosome number, with *S. comptum* being tetraploid ($n = 24$) and *S. hieronymi* having a variety of ploidy levels ($n = 12$, 18, and 24; Chiarini 2007). Similar to the North American species in the Carolinense clade, these four South American species are also weedy and are commonly found in disturbed areas such as roadsides, fields, pastures, and waste areas, as well as in arid vegetation types.

Solanum moxosense was described by Nee in 2006 (Nee et al. 2006) and is known only from the type locality in Beni Department, Bolivia where it was characterized as having a creeping habit and stems that often root at the nodes. The species was placed in *S.* subsect. *Lathyrocarpum* by Nee (1999) as '*S.* sp. nov. ined, *M. Nee 34261*', and the results presented here showed the species to be consistently resolved in the Carolinense clade (Fig. 1; Table 1). *Solanum reineckii* is restricted to southern Brazil and is similar to *S. moxosense* in its creeping habit and stems that root at the nodes. In the analysis of the combined data set, *S. reineckii* and *S. moxosense* were resolved as successive sister taxa to the other South American species (Fig. 1), a relationship that may suggest the creeping habit and stems rooting at the nodes are ancestral states in the Carolinense clade.

Putative Allopolyploidy and Hybridization in the Carolinense Clade—It has been inferred that the base chromosome number in *Solanum* subg. *Leptostemonum* is $x = 12$ (Chiarini and Bernardello 2006, and citations therein). One of the earliest reports of polyploidy in the spiny solanums was $n = 36$ for *S. perplexum* (as *S. dimidiatum*; D'Arcy 1969). Since then, chromosome numbers have been published for several species in the Carolinense clade ($n = 12$ for *S. aridum* and *S. carolinense*; $n = 24$ for *S. comptum* and *S. juvenale*; $n = 36$ for *S. dimidiatum* and *S. perplexum*; and $n = 12$, 18, and 24 for *S. hieronymi*; D'Arcy 1969; Nichols and Hanna 1984; Moscone 1992; Chiarini and Bernardello 2006; Chiarini 2007). In this study, three species with high or variable ploidy levels (*Solanum dimidiatum*, *S. perplexum*, and *S. hieronymi*) were placed in different clades depending on the analysis of the nuclear vs. plastid data set (Fig. 2). These alternate phylogenetic placements are consistent with speciation involving allopolyploidization or hybridization. Also, since multiple accessions were sequenced for the three species, the conflicting relationships suggest a reticulating evolutionary history and not an artifact of sequencing or analysis.

The molecular phylogenetic evidence presented here, along with the hexaploid chromosome counts for *Solanum dimidiatum* and *S. perplexum*, points to an allopolyploid origin for these two species. Additional evidence comes from the polymorphic nucleotide positions seen in the electropherograms of *waxy* sequences for both species in this study. Polymorphisms are commonly seen where different alleles are maintained in the separate parental nuclear genomes of polyploid species (e.g. Ramsey and Schemske 1998). An allopolyploid origin could be hypothesized via an ancient hybridization between *S. dimidiatum* or *S. perplexum* as the pollen donor and a species from the Torva clade as the female parent. A problem with this scenario, however, is that there are no obvious candidate parental species from the Torva clade in North America.

The differing pattern of phylogenetic relationships and variable ploidy levels of *Solanum hieronymi* also suggest a speciation history involving hybridization or introgression. Based on the phylogenetic reconstruction in this study, a possible scenario of hybrid origin would involve a member of the Elaeagnifolium clade as the pollen donor (several species of which co-occur with *S. hieronymi*) and a South American species from the Carolinense clade as the female parent (e.g. *S. comptum*). However, there is insufficient resolution in the phylogeny to infer which parental species may have been involved. Also, the ploidy levels of the *S. hieronymi* accessions used in this study are not known; inclusion of *S. hieronymi* samples with variable ploidy levels may suggest a more complicated scenario.

The phylogenetic results presented in this study are consistent with a reticulating evolutionary history involving three species in the Carolinense clade (*Solanum dimidiatum*, *S. perplexum*, and *S. hieronymi*) and other closely related spiny solanums. However, to confirm and reconstruct the hybrid origin of these species, additional nuclear and plastid DNA sequence data and denser taxon sampling of closely related clades are needed to improve resolution so that parental species can be identified. Studies of chromosome numbers and experimental crosses in the greenhouse would add additional evidence to infer the origins and relationships of these three putatively hybrid-derived species.

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APPENDIX 1. List of taxa and voucher specimens used in this study (taxon, provenance, collector, collection number, and herbarium where the voucher is deposited). GenBank accession numbers given in the order: ITS, waxy, and trnT−trnF.

Solanum 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. 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. arboreum*** Dunal. COSTA RICA. *Bohs 2521* (UT) AF244719, AY996381, DQ180424. ***S. argentinum*** Bitter & Lillo. ARGENTINA. *Bohs 2539* (UT) AF244718, AY996382, DQ180425. ***S. aridum*** (1) Morong. BOLIVIA. *Bohs & Nee 2733* (UT) AY996499, AY996400, DQ180479. ***S. aridum*** (2). ARGENTINA. *Chiarini & Wahlert 905* (CORD) KC539138, KC539183, KC539160. ***S. aridum*** (3). BOLIVIA. *Wood & Goyder 15726* (K) KC539143, KC539188, KC539165. ***S. asterophorum*** Mart. BRAZIL. *Agra et al. 7010* (JPB) GU591059, GU591111, GU591000. ***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. 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. carolinense*** var. ***carolinense*** L. (1). U. S. A. BIRM S.1816, *Olmstead S-77* (WTU) AY996491, AY996392, DQ180476. ***S. carolinense*** var. ***carolinense*** (2). U. S. A. *Diamond 16776* (UNA) KC539137, KC539184, KC539161. ***S. carolinense*** var. ***carolinense*** (3). U. S. A. *Jones 27-06* (MO) KC539141, KC539186, KC539163. ***S. carolinense*** var. ***carolinense*** (4). U. S. A. *Spongberg et al. 17119* (MO) KC539142, KC539187, KC539164. ***S. carolinense*** var. ***floridanum*** Chapm. (1). U. S. A. *Anderson 23263* (FSU) KC539139, KC539193, KC539159. ***S. carolinense*** var. ***floridanum*** (2). U. S. A. *Scanlon & Matthews 138* (FLAS) KC539140, KC539185, KC539162. ***S. cinereum*** R. Br. NIJ 904750120, *Bohs 2852* (UT) AY996493, AY996394, DQ180397. ***S. comptum*** C.V. Morton (1). ARGENTINA. *Barboza et al. 2078* (CORD) KJ145735, KC539202, KC539181. ***S. comptum*** (2). ARGENTINA. *Chiarini & Wahlert 832* (CORD) KC539145, KC539190, KC539167. ***S. comptum*** (3). PARAGUAY, *Bohs 3203* (UT) KC539144, KC539189, KC539166. ***S. comptum*** (4). PARAGUAY. *Bohs 3193* (UT) AY996498, AY996399, GU591009. ***S. cordovense*** Sessé & Moç. COSTA RICA. *Bohs 2693* (UT). AF244717, AY996401, DQ180480. ***S. crinitipes*** Dunal. COLOMBIA. *Olmstead S-81* (WTU) AY996500, AY996402, DQ180481. ***S. crinitum*** Lam. BRAZIL. *Agra et al. 7028* (JPB) GQ143651, GQ143683, GQ149736. ***S. crotonoides*** Lam. DOMINICAN REPUBLIC. *Nee 52298* (NY) AY996502, AY996404, GU591011. ***S. dimidiatum*** Raf. (1). U. S. A. *Quayle 1005* (BRIT) KJ145737, KJ145728, KJ145745. ***S. dimidiatum*** (2). U. S. A. *O'Kennon & McLemore 19532* (BRIT) KJ145738, KJ145727, KJ145744. ***S. dimidiatum*** (3). U. S. A. *Mazer et al. 5* (FLAS) KC539146, —, KC539168. ***S. dimidiatum*** (4). U. S. A. *Ricketson et al. 4758* (BRIT) KJ145736, KJ145726, KJ145743. ***S. diploconos*** (Mart.) Bohs. BRAZIL. *Bohs 2335* (UT) AY523890, AY996407, DQ180429. ***S. donianum*** Walp. MEXICO. *Bohs 3472* (UT) GU591069, GU591121, GU591013. ***S. dulcamara*** L. cultivated, U. S. A. no voucher; AF244742, AY996410, AY226231. ***S. elaeagnifolium*** Cav. (1). PARAGUAY. *Bohs 3204* (UT) AY996508, AY996412, DQ180399. ***S. elaeagnifolium*** (2). U. S. A. *Olmstead S-82* (WTU) AF244730, AY996413, GU591014. ***S. elaeagnifolium*** (3). ARGENTINA. *Chiarini & Wahlert 879* (CORD) KC539148, KC539191, KC539170. ***S. ensifolium*** Dunal. PUERTO RICO. *Bohs 2461* (UT) AY996506, AY996409, DQ180483. ***S. euacanthum*** Phil. ARGENTINA. *Barboza 2131* (CORD) KC539149, KC539192, KC539171. ***S. furfuraceum*** R. Br. BIRM S.1442, *Olmstead S-84* (WTU) AY996512, AY996417, DQ180401. ***S. glaucophyllum*** Desf. No voucher; AF244714, AY996418, DQ180430. ***S. glutinosum*** Dunal. NIJ A34750191, *Bohs 3262* (UT) AY996513, AY996419, GU591016. ***S. hasslerianum*** Chodat. (1). ARGENTINA. *Barboza et al. 2121* (CORD) GU591071, GU591123, GU591017. ***S. hasslerianum*** (2). ARGENTINA. *Barboza et al. 1530* (CORD) KC539150, KC539201, KC539172. ***S. hieronymi*** Kuntze. (1). ARGENTINA. *Nee & Bohs 50761* (NY) AY996517, AY996423, GU591019. ***S. hieronymi*** (2). ARGENTINA. *Chiarini & Wahlert 897* (CORD) KC539152, KC539195, KC539174. ***S. hieronymi*** (3). BOLIVIA. *Mendoza & Calzadilla 765* (K) KC539151, KC539194, KC539173. ***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. homalospermum*** Chiarini. ARGENTINA. *Chiarini 566* (CORD) KC539153, KC539196, KC539175. ***S. jamaicense*** Mill. BIRM S.1209, *Olmstead S-85* (WTU) AF244724, AY562956, DQ180485. ***S. juvenale*** Thell. ARGENTINA. *Barboza et al. 2071* (CORD) GU591074, GU591126, GU591021. ***S. laciniatum*** Aiton. NEW ZEALAND. *Bohs 2528* (UT) AF244744, AY996431, DQ180467. ***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. lycocarpum*** A. St.-Hil. PARAGUAY. *Bohs 3212* (UT) AY996525, AY996435, DQ812107. ***S. melongena*** L. BIRM S.0657, *Olmstead S-91* (WTU) GU591078, AY562959, DQ180406. ***S. microphyllum*** (Lam.) Dunal. DOMINICAN REPUBLIC. *Nee 52300* (NY) AY996529, AY996441, GU591026. ***S. mitlense*** Dunal. MEXICO. *Whalen & Velasco 825* (BH) AY996530, AY996442, DQ812108. ***S. mortonii*** Hunz. ARGENTINA. *Barboza et al. 639* (CORD) GU591080, GU591131, GU591028. ***S. moxosense*** M. Nee. (1). BOLIVIA. *McClelland & Stern 408* (NY) GU591081, GU591132, GU591029. ***S. moxosense*** (2). BOLIVIA. *Nee 34261* (NY) KJ145741, KJ14572, KJ145746. ***S. multispinum*** N. E. Br. (1). PARAGUAY. *Bohs 3198* (UT) AY996533, AY996444, GU591030. ***S. multispinum*** (2). PARAGUAY. *Peña-Chocarro & Knapp 1485* (BM) KC539154, KC539197, KC539176. ***S. multispinum*** (3). ARGENTINA, *Chiarini & Wahlert 888* (CORD) KC539155, KC539198, KC539177.

S. nemorense Dunal. BOLIVIA. *Bohs & Nee 2757* (UT) AY996536, AY996447, DQ180488. ***S. palinacanthum*** Dunal. BOLIVIA. *Bohs 3151* (UT) AY561268, AY562961, AY266233. ***S. paniculatum*** L. PARAGUAY. *Bohs 3181* (UT) AY996540, AY996452, GU591032. ***S. paraibanum*** Agra. BRAZIL. *Agra et al. 7008* (JPB) GU591083, GU591134, GU591033. ***S. perplexum*** Small. (1). U. S. A. *MacDonald 10468* (FSU) KJ145742, KJ145730, KJ145747. ***S. perplexum*** (2). U. S. A. *Diamond 13245* (FSU) KJ145734, KJ145731, KJ145748. ***S. perplexum*** (3). U. S. A. *Tomlinson s. n.* (FLAS) KC539147, —, KC539169. ***S. piluliferum*** Dunal. BRAZIL. *Agra et al. 7295* (JPB) HQ457398, HQ457417, HQ457407. ***S. pseudocapsicum*** L. BIRM S.0870, no voucher; AF244720, AY562963, DQ180436. ***S. ptychanthum*** Dunal. U. S. A. Chicago, *Olmstead S-94* (WTU) AF244735, AY996457, DQ180454. ***S. pumilum*** Dunal. (1). U. S. A. *Allison & Stevens 8241* (UNA) KC539156, KC539199, KC539178. ***S. pumilum*** (2). U. S. A. *Allison & Stevens 7644* (UNA) KC539157, KC539200, KC539179. ***S. refractum*** Hook. & Arn. MEXICO. *Iltis et al. 29694* (WIS) AY996547, AY996460, HQ457408. ***S. reineckii*** Briq. (1). BRAZIL. *Metzenbacher s. n.* (UT) KJ145739, KJ145732, KJ145749. ***S. reineckii*** (2). BRAZIL. *Mentz et al. 408* (UT) KJ145740, KJ145733, KJ145750. ***S. rostratum*** Dunal. NIJ 934750126, *Cipollini 173* (UT) GQ143670, GQ143702, GQ149755. ***S. rupincola*** Sendtn. BRAZIL. *Thomas et al. 1571* (MO) GU591091, GU591142, GU591041. ***S. sisymbriifolium*** Lam. (1). ARGENTINA. *Bohs 2533* (UT) AY561271, AY562967, AY266235. ***S. sisymbriifolium*** (2). ARGENTINA. *Chiarini & Wahlert 884* (CORD) KC539158, KC539182, KC539180. ***S. stagnale*** Moric. BRAZIL. *Bohs 3094* (UT) GU591093, GU591144, GU591043. ***S. stenandrum*** Sendtn. BRAZIL. *Irwin et al. 33085* (WIS) AY561273, AY562969, AY559242. ***S. stramonifolium*** Jacq. PERU. *Whalen 860* (HUT) AY263465, AY562970, AY266263. ***S. thomasiifolium*** Sendtn. BRAZIL. *Tavares et 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. viarum*** Dunal. NIJ 934750190, *Cipollini 67* (UT) AY561275, AY562973, AY559243. ***S. wendlandii*** Hook. f. BIRM S.0488, no voucher; AF244731, AY562974, DQ180440.