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A FOUR-GENE STUDY OF EVOLUTIONARY RELATIONSHIPS IN *SOLANUM* SECTION *ACANTHOPHORA*[1]

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The ''spiny solanums,'' *Solanum* subgenus *Leptostemonum* (Solanaceae), comprise a large lineage with over 350 species and include the cultivated eggplant, *Solanum melongena*. Despite the importance of this subgenus, phylogenetic relationships among these taxa are currently unclear. The present research contributes to this understanding while focusing on *Solanum* section *Acanthophora*, a group of ca. 19 species defined by the presence of simple hairs, rather than the stellate hairs common across the rest of subgenus *Leptostemonum*. In this study we inferred phylogenetic relationships among 29 *Solanum* taxa, including 14 species of section *Acanthophora*, using DNA sequence data from two nuclear regions (ITS and the granule-bound starch synthase gene [GBSSI or *waxy*]) and two chloroplast regions (*trnT-trnF* and *trnS-trnG*). This combination of gene regions resulted in a well resolved phylogenetic hypothesis, with results strongly suggesting that *Solanum* sect. *Acanthophora* is not monophyletic, although the majority of taxa comprise a monophyletic lineage that is sister to *Solanum* section *Lasiocarpa*. Of the four gene regions, *waxy* was especially useful for phylogenetic inference, with both a high percentage of parsimony-informative sites as well as a low level of homoplasy. Further studies in progress will help elucidate relationships of sect. *Acanthophora* with respect to other members of subgenus *Leptostemonum*.

Key words: *Acanthophora*; GBSSI; ITS; Solanaceae; *Solanum*; *trnS-trnG*; *trnT-trnF*; *waxy*.

Solanum subgenus *Leptostemonum* (Dunal) Bitter, with approximately 350–450 species, is the largest subgenus of the enormous genus *Solanum*. Members of this subgenus are also referred to as ''spiny solanums,'' because the majority of species are armed with epidermal prickles. Whereas most taxa within subgenus *Leptostemonum* bear stellate hairs, species of *Solanum* section *Acanthophora* Dunal are unusual in having pubescence of largely simple hairs. Exclusively unbranched hairs are found on the stems and upper leaf surfaces of most species in sect. *Acanthophora*, whereas the undersurfaces may have mixed stellate and simple hairs. These plants are among the most ferocious of the spiny solanums, with their needle-like prickles bristling from all vegetative parts (Fig. 1).

Solanum section *Acanthophora* includes about 20 mainly neotropical species of herbs and small shrubs. This section is synonymous with the *Solanum mammosum* species group of Whalen (1984). Nee (1979a) monographed section *Acanthophora* and included 19 species, with two unpublished species now considered synonyms of other names (M. Nee, New York Botanical Garden, personal communication). In addition, the species of the *S. wacketii* group of Whalen (1984) are now considered to be closely related to or included in section *Acanthophora* (M. Nee, personal communication). Whalen (1984) suggested a close connection between the *S. mammosum* and *S. wacketii* groups due in part to their shared pubescence of simple hairs on the stems. Species of the *S. wacketii* group, however, are distinguished by their stellate leaf pubescence and petaloid calyces.

The center of diversity of section *Acanthophora* is eastern Brazil, where all but three of the species are native (Nee, 1979a; Table 1). Most are adapted to disturbed habitats and secondary forest in open, sunny situations. A number of species in the section have been introduced and naturalized in other parts of the Americas and in the Old World (Table 1); a few, such as *S. viarum*, have become noxious weeds in areas outside their native ranges (e.g., Wunderlin et al., 1993). Several species are widely cultivated for their ornamental fruits (e.g., *S. mammosum*, *S. capsicoides*) or for their alkaloid content (*S. mammosum*, *S. viarum*).

Section *Acanthophora* has been divided into three subsections mainly based upon fruit and seed characters (Nee, 1979a). Because two of these subsectional names have not been published, the three groups are referred to by informal names in this paper (Table 1). Species of the Pterosperma group are distinguished by their highly flattened seeds with a prominent wing. Species of the other two groups lack this marginal wing. The monotypic Oligosperma group is distinctive in its diminutive stature and few- (1–3) seeded fruits. The Euleptostemonum group includes the remainder of the species in the section, characterized by many-seeded fruits and lack of a seed wing.

Species of section *Acanthophora* exhibit great variation in their fruit morphology. *Solanum palinacanthum* and *S. mammosum* produce the largest fruits in the section, reaching ca. 3.5–5.5 cm in diameter, and the fruits of *S. mammosum* are some of the most bizarre of any species of *Solanum*. Some *S. mammosum* plants produce globose fruits, but better known are the forms with large, elongated, nipple-shaped fruits up to 8 cm long, usually subtended by five protuberances. Both of these species have a whitish, spongy mesocarp layer that can be up to 7 mm thick. The function of this spongy layer is unknown; it has been suggested that these fruits are adapted for dispersal by floating or, alternatively, are adapted for resistance to fire (Nee, 1979a). Nee (1979a) found that fruits of *S. mammosum* can float in water for nearly one year and spec-

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Fig. 1. *Solanum acerifolium* showing the extensive vegetative prickles typical of section *Acanthophora*. Photo courtesy of J. S. Miller, Amherst College, Massachusetts, USA, and P. K. Diggle, University of Colorado, Boulder, Colorado, USA.

ulated that this may be an adaptation to dispersal in the seasonally flooded llanos of Venezuela and Colombia, where he presumes this species to be native. *Solanum palinacanthum* often occurs in pastures, and the fruits may be eaten by cattle (M. Nee, personal communication).

Solanum capsicoides and *S. platense* have somewhat smaller fruits about 2–3 cm in diameter. The fruits of *S. capsicoides* turn bright orange at maturity and have abundant white, spongy mesocarp as in *S. mammosum* and *S. palinacanthum*. Nee (1991) observed that fruits of *S. capsicoides* and *S. platense* split irregularly at maturity and, thus, may form a shaker or censer mechanism to disperse the seeds. *Solanum viarum*, *S. myriacanthum*, and *S. aculeatissimum* also have relatively large fruits ca. 2–3 cm in diameter that turn yellow at maturity. *Solanum viarum* (and perhaps the other species in the *S. viarum–S. myriacanthum–S. aculeatissimum* complex) has a thick spongy mesocarp as in the other large-fruited species (L. Bohs, personal observation). Dispersal agents for these species are unknown, but Nee (1979a) speculates that they may be mammal-dispersed. In Florida, where *S. viarum* has become a weed, cattle occasionally eat the fruits and the seeds are spread among pastures in hay and farm implements (Wunderlin et al., 1993). *Solanum viarum* fruits are also buoyant and may be dispersed by water (Bryson and Byrd, 1994).

Nearly all the other species in the section have globose fruits approximately 1–1.5 cm in diameter, commonly light green with darker green stripes or mottling and turning yellow or orange when mature. These species have sparse and sticky mesocarp and are presumably bird dispersed (Nee, 1979a, 1991). *Solanum tenuissimum* is distinguished from the other species in the section by its very small (4–5 mm diameter) fruits with 1–3 seeds per fruit. These characters are so unique within the section that Nee (1979a) placed *S. tenuissimum* in the monotypic Oligosperma group.

Several species of section *Acanthophora* have very high levels of fruit glycoalkaloids, accounting for their folk uses as poisons to kill rats or cockroaches (Nee, 1979a, b). The fruits of *S. mammosum* are widely used as toxins to kill pests, as fish poisons, as medicines, and in fertility rites due to their unusual shape (Nee, 1979b). *Solanum capsicoides* fruits are considered to be poisonous in Edo. Paraíba, Brazil (M. Agra, Universidade Federal da Paraíba, personal communication), and some common names applied to this species (''cockroach poison,'' ''poison diable'') apparently refer to its toxic properties. In Uganda, the fruits of *S. aculeatissimum* are believed to be toxic, and the plants are used in witchcraft (Bukenya and Carasco, 1999). *Solanum viarum* has been investigated in India as a source of steroidal alkaloids (Nee, 1991). Solasodine, a steroidal alkaloid common in many *Solanum* species, has been isolated from fruits of *S. mammosum*, *S. atropurpureum*, *S. acerifolium*, *S. viarum*, and perhaps *S. myriacanthum* (Nee, 1979a). Solasodine is concentrated in the fruits (especially unripe fruits), with little or no alkaloid in other parts of the plant, including the seeds (Nee, 1991). Because of its diversity in fruit types and glycoalkaloid content, section *Acanthophora* is an ideal group for the investigation of fruit morphology and chemistry in a phylogenetic and ecological context (Cipollini et al., 2002).

Although Nee (1979a) previously monographed *Solanum* section *Acanthophora*, to date there have been no molecular phylogenetic studies of the group. Thus, the main goal of the present study is to examine phylogenetic relationships among species of section *Acanthophora*, with sufficient sampling to evaluate its monophyly, as well as to determine its component clades. Understanding the evolutionary history of these taxa will facilitate the examination of fruit evolution within the section, including patterns of fruit morphology and glycoalkaloid content.

In this paper we (1) examine phylogenetic relationships within section *Acanthophora* and among the section and closely related members of subgenus *Leptostemonum*, (2) test the monophyly of section *Acanthophora* as currently circumscribed, (3) evaluate the utility of four different gene regions (two nuclear and two chloroplast) in resolving relationships among closely related *Solanum* species, and (4) explore character evolution (particularly fruit morphology and chemistry) in the context of evolutionary relationships within section *Acanthophora*. Phylogenetic relationships are inferred from DNA sequence data of four gene regions. These regions include two from the nuclear genome: the nuclear ribosomal internal transcribed spacer region (ITS) and the granule-bound starch synthase (GBSSI) or *waxy* gene (van der Leij et al.,

TABLE 1. Species of *Solanum* section *Acanthophora* and their distributions according to Nee (1979a, 1979b). Asterisks indicate species included in this study.

Solanum sect. *Acanthophora* (Dunal) Bitter	Geographic distributions
Pterosperma group	
S. acerifolium Humb. & Bonpl. ex Dunal*	Mexico to Panama, Andes (Ecuador to Venezuela), scattered in eastern Brazil and eastern Paraguay
S. affine Sendtn.	Southeastern Brazil
S. atropurpureum Schrank*	Native to northeastern Argentina, Paraguay, Uruguay, southeastern Brazil; introduced to Colombia
S. capsicoides All.*	Native to east coast of Brazil; introduced to North and Central America, northern South America, Caribbean, Old World
S. incarceratum Ruiz & Pav.*	Peru, Bolivia, Paraguay, eastern Brazil
S. platense Dieckmann*	Eastern Argentina, Uruguay, southeastern Brazil
S. tejucense Dunal	Southeastern Brazil
S. tenuispinum Rusby* (syn. *S. chaetophorum* C. V. Morton)	Andes (southern Peru, Bolivia, northwest Argentina)
S. vaillantii Dunal*	Southeastern Brazil
Species A (M. Nee, ined.)	Southeastern Brazil
Species B (M. Nee, ined.)	Known from two collections: southeastern Brazil and Mérida, Venezuela
Oligosperma group	
S. tenuissimum Sendtn.	Planalto of Goiás, Brazil
Euleptostemonum group	
S. aculeatissimum Jacq.*	Southeastern Brazil, Africa; sporadic in Asia
S. agrarium Sendtn.*	Northeastern Brazil (coastal), Venezuela, Colombia, Caribbean
S. mammosum L.*	Native to northern South America and Caribbean; naturalized in Andes from Peru north to Central America and Mexico, West and East Indies, Brazil, and Old World
S. myriacanthum Dunal*	Mexico south to Nicaragua; sporadic in Cuba and USA
S. palinacanthum Dunal*	Brazil, Paraguay, Argentina, Bolivia
S. stenandrum Sendtn.*	Northeastern Brazil (inland)
S. viarum Dunal*	Brazil, Argentina, Paraguay, Uruguay; naturalized in North America and Old World

1991; Mason-Gamer et al., 1998; Peralta and Spooner, 2001). Chloroplast data are from the *trnT-trnF* (Taberlet et al., 1991) and *trnS-trnG* (Hamilton, 1999) spacer regions.

MATERIALS AND METHODS

Taxon sampling—The majority of species (14 of 19 species) in section *Acanthophora* sensu Nee (1979a) were sampled for this study (Table 1). Chromosome counts have been reported for six of the sampled *Acanthophora* species, and all are diploids with $2n = 22$ (*S. mammosum*) or $2n = 24$ (*S. atropurpureum*, *S. capsicoides*, *S. myriacanthum*, *S. palinacanthum*, and *S. viarum*; Goldblatt and Johnson, 2004). We were unable to obtain the high quality genomic DNA needed for five species in sect. *Acanthophora*, nor were we able to sample from the *S. wacketii* group of Whalen (1984), which is thought to be closely related to sect. *Acanthophora* (M. Nee, New York Botanical Garden, personal communication). In addition to the 14 species sampled from sect. *Acanthophora*, we have included a broad sampling of *Solanum* relatives as outgroups. These included 11 other species in *Solanum* subgenus *Leptostemonum* chosen to represent a variety of diverse clades based on previous analyses, including four species in *Solanum* sect. *Lasiocarpa*, which emerged as the sister group to sect. *Acanthophora* with 100% bootstrap support in a recent study based on cpDNA sequence data (Bohs, 2004). Sampling also included four taxa from various *Solanum* clades outside of subgenus *Leptostemonum*. All 29 taxa with voucher information and GenBank accession numbers are listed in Appendix 1 (see Supplemental Data accompanying the online version of this article).

DNA extraction, amplification, and sequencing—Total genomic DNA was extracted from fresh, silica gel-dried, or herbarium material using the protocols described in Bohs and Olmstead (1997, 2001) and Bohs (in press).

ITS—Amplification of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA, composed of ITS1, the 5.8S gene, and ITS2 (Baldwin, 1992; Baldwin et al., 1995) was done using primers ITSleu1 (5′-GTC CAC TGA ACC TTA TCA TTT AG-3′; Bohs and Olmstead, 2001) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′; White et al., 1990). Polymerase chain reaction (PCR) conditions were as in Bohs and Olmstead (2001), and PCR products were cleaned using the QIAquick PCR purification kit (Qiagen, Valencia, California, USA). Sequencing was done in both directions on an ABI automated sequencer (Applied Biosystems, Foster City, California, USA) by the University of Utah DNA Sequencing Core Facility using ITS4 and ITS5HP (5′-GGA AGG AGA AGT CGT AAC AAG G-3′; Hershkovitz and Zimmer, 1996).

Waxy—Amplification of the 3′ end of exon 1 through the 3′ end of exon 10 of the nuclear GBSSI gene was done using primers 5′old (5-GGG TAC TGA GGT TGG TCC TT-3′; D. M. Spooner, U.S. Department of Agriculture, University of Wisconsin, Madison) and 2R (5′-GTT CCA TAT CGC ATA GCA TG-3′; in Miller et al. [1999] as 5′-GTT CCA TAC GCA TAG CAT G-3′, with an omission of a base at the ninth position) (Fig. 2). Reactions of 25 μL were done containing 2.5 μL 10× Mg-free buffer, 2.5 mmol/L $MgCl_2$, 0.25 mmol/L dNTPs, 0.08 μmol/L of each primer, 0.625 units of *Taq* polymerase, and 1 μL DNA. The thermal cycler program was an initial 1 min at 94°C; 5 cycles at 94°C for 45 s, 60°C for 2 min, and 72°C for 1 min; 30 cycles at 94°C for 30 s, 60°C for 40 s, 72°C for 1 min; ending with an extension at 72°C for 20 min. Occasionally amplifications were done with an alternate forward primer, 181F (5′-CGG GTA ATG ACA ATA TST CC-3′; Walsh and Hoot, 2001), which primes at the 5′ end of exon 2. With this primer, a different set of thermal cycler conditions was used: initial denaturing for 4 min at 94°C; 5 cycles of 94°C for 30 s, 50°C for 1 min, and 72°C for 1 min 30 s; 30 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min 30 s; ending with a final extension at 72°C for 10 min. The PCR products were cleaned and sequenced using primers 181F (or rarely 5′old) and 2R, as well as internal primers 1171R (5′-TCA TAC CCA TCA ATG AAA TC-3′; Walsh and Hoot, 2001), 3F (5′-GAT ACC CAA GAG TGG AAC CC-3′;

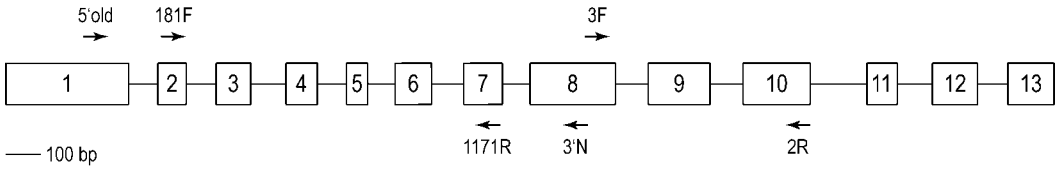

Fig. 2. Diagram of the granule-bound starch synthase (GBSSI, *waxy*) gene, with the locations of the amplification and sequencing primers used in this study. The sequences from this study include the region between the primers 181F and 2R. Boxes indicate exons. Exon 1 is preceded by an untranslated exon, which is not shown.

Miller et al., 1999), and occasionally 3′ N (5′-GCC ATT CAC AAT CCC AGT TAT GC-3′; Peralta and Spooner, 2001) (Fig. 2).

trnT-trnF—We amplified the entire chloroplast region between the *trnT* and *trnF* genes, including the intergenic spacer between *trnT* and *trnL,* the *trnL* 5′ and 3′ exons, the *trnL* intron, and the intergenic spacer between the *trnL* 3′ exon and *trnF*. Primers used for amplification were a and f (Taberlet et al., 1991), in 25 μL reactions as described in Bohs and Olmstead (2001) and Bohs (2004). For *S. atropurpureum* and *S. stenandrum*, amplification was done in two reactions using primers a and d and primers c and f (Taberlet at al., 1991). The PCR products were cleaned and sequenced as above using primers a through f (Taberlet et al., 1991).

trnS-trnG—The chloroplast intergenic spacer between *trnS* and *trnG* was amplified using primers trn S (5′-GCC GCT TTA GTC CAC TCA GC-3′) and trn G (5′-GAA CGA ATC ACA CTT TTA CCA C-3′) of Hamilton (1999). Reactions of 25 μL were done, with 2.2 μL Mg-free 10X buffer, 2.2 mmol/L $MgCl_2$, 0.25 mmol/L dNTPs, 0.36 μmol/L of each primer, 0.625 units of *Taq* polymerase, and 1 μL DNA. The thermal cycler program included an initial denaturing at 94°C for 4 min; 40 cycles at 94°C for 45 s, 52°C for 1 min, 72°C for 1 min; ending with an extension at 72°C for 7 min. The PCR products were cleaned and sequenced as above using the same primers as for amplification.

Sequence alignment—Sequences were edited, and a consensus sequence for each species was constructed using Sequencher (Gene Codes Corporation, Ann Arbor, Michigan, USA). Species sequences were then aligned manually in SeAl (Rambaut, 1996) and MacClade 4.0 (Maddison and Maddison, 2000). The alignments of all four gene regions are available on TreeBASE.

Phylogenetic analyses—The four data sets were analyzed separately (Table 2) and in various combinations with other data sets (see below). Parsimony analyses were conducted in PAUP* 4.0b10 (Swofford, 2002) using branch and bound searches with the MulTrees option in effect (*trnT-trnF, waxy,* all data sets) or heuristic searches with 500 random addition sequence replicates and the MulTrees option in effect (ITS, *trnS-trnG,* ITS + *waxy, trnT-trnF* + *trnS-trnG*). Gaps were treated as missing data. The strength of support for individual tree branches was estimated using bootstrap values (BS) (Felsenstein, 1985) and decay indices (DI) (Bremer, 1988; Donoghue et al., 1992). Bootstrap values were from 500 full heuristic bootstrap replicates, each with 10 random addition sequence replicates. The MulTrees option was not in effect for the two chloroplast data sets. Decay values for each branch were determined using the PAUP decay index command file in MacClade to prepare a set of trees each with a single branch resolved. To find the shortest trees consistent with each constraint, this file was executed in PAUP* using the heuristic search option with 100 random addition sequence replicates and the MulTrees option in effect. The decay index for each branch is the difference in length between the shortest trees consistent with each constraint and the globally shortest trees.

In all analyses, trees were rooted with *S. aviculare*, as previous studies strongly suggested that *S. aviculare* is a basal taxon within *Solanum* (e.g., Bohs and Olmstead, 2001). Due to missing data for two or three gene regions, *Solanum vaillantii* was excluded from all analyses except *trnS-trnG* only and *trnT-trnF* + *trnS-trnG,* and *S. agrarium* was excluded from all analyses except ITS only, *trnS-trnG* only, ITS + *waxy,* and *trnT-trnF* + *trnS-trnG*. Congruence of the data sets (excluding these two taxa) was tested using partition homogeneity tests (incongruence length difference test [ILD]; Farris et al., 1994, 1995) as implemented in PAUP*. One thousand heuristic partition homogeneity replicates were completed, each with 10 random addition sequence replicates, tree bisection-reconnection (TBR) branch-swapping, MulTrees off, and gaps treated as missing data.

Maximum likelihood—An analysis using a maximum likelihood (ML) model was conducted with all four data sets combined. The ML model parameters were determined using Modeltest version 3.06 (Posada and Crandall, 1998). This program tests the fit of 56 substitution models to the data; based on a hierarchical likelihood ratio test, a model that best fits the data is identified. The best model was used in a ML analysis in PAUP* using the heuristic search option, all most-parsimonious trees from the parsimony analysis of the four combined data sets as the starting trees, TBR branch-swapping, and the MulTrees option in effect. As in the parsimony analysis of the four data sets combined, *S. agrarium* and *S. vaillantii* were excluded, and *S. aviculare* was defined as the outgroup.

Alternate topologies—A constraint tree was constructed in MacClade to test the monophyly of *Solanum* sect. *Acanthophora*. This tree was loaded into PAUP*, and a heuristic search was conducted to find the shortest trees consistent with the constraint. The number of additional steps required for the constraint is the difference between the shortest trees consistent with the constraint and the globally shortest trees. Further, a one-tailed nonparametric Shimodaira-Hasegawa test (S-H test; Shimodaira and Hasegawa, 1999; see also Goldman et al., 2000) was conducted in PAUP* to assess the statistical support for this constraint, using the same ML parameters outlined above. In this procedure, the likelihoods of all four shortest trees constrained to contain the lineage of interest were compared with the likelihood of a random most-parsimonious tree (MPT) from the unconstrained analysis. Resampling esti-

TABLE 2. Comparison of the 27 taxa data sets for the two nuclear and two chloroplast regions.

Statistic	ITS	*waxy*	*trnT-trnF*	*trnS-trnG*
Range of raw length	614–652 bp	1785–1815 bp	1745–2046 bp	656–708 bp
Aligned length	676 bp	1831 bp	2397 bp	819 bp
Variable sites (proportion)	202 (0.30)	397 (0.22)	261 (0.11)	109 (0.13)
PI sites (proportion)	110 (0.163)	139 (0.076)	82 (0.034)	27 (0.033)
Range of pairwise distances	0.008–0.129	0.002–0.062	0.001–0.033	0–0.039
CI (RC); RI	0.62 (0.42); 0.67	0.86 (0.72); 0.84	0.88 (0.77); 0.87	0.87 (0.74); 0.85

Note: Parsimony-informative = PI; consistency index = CI (RC = rescaled CI); retention index = RI.

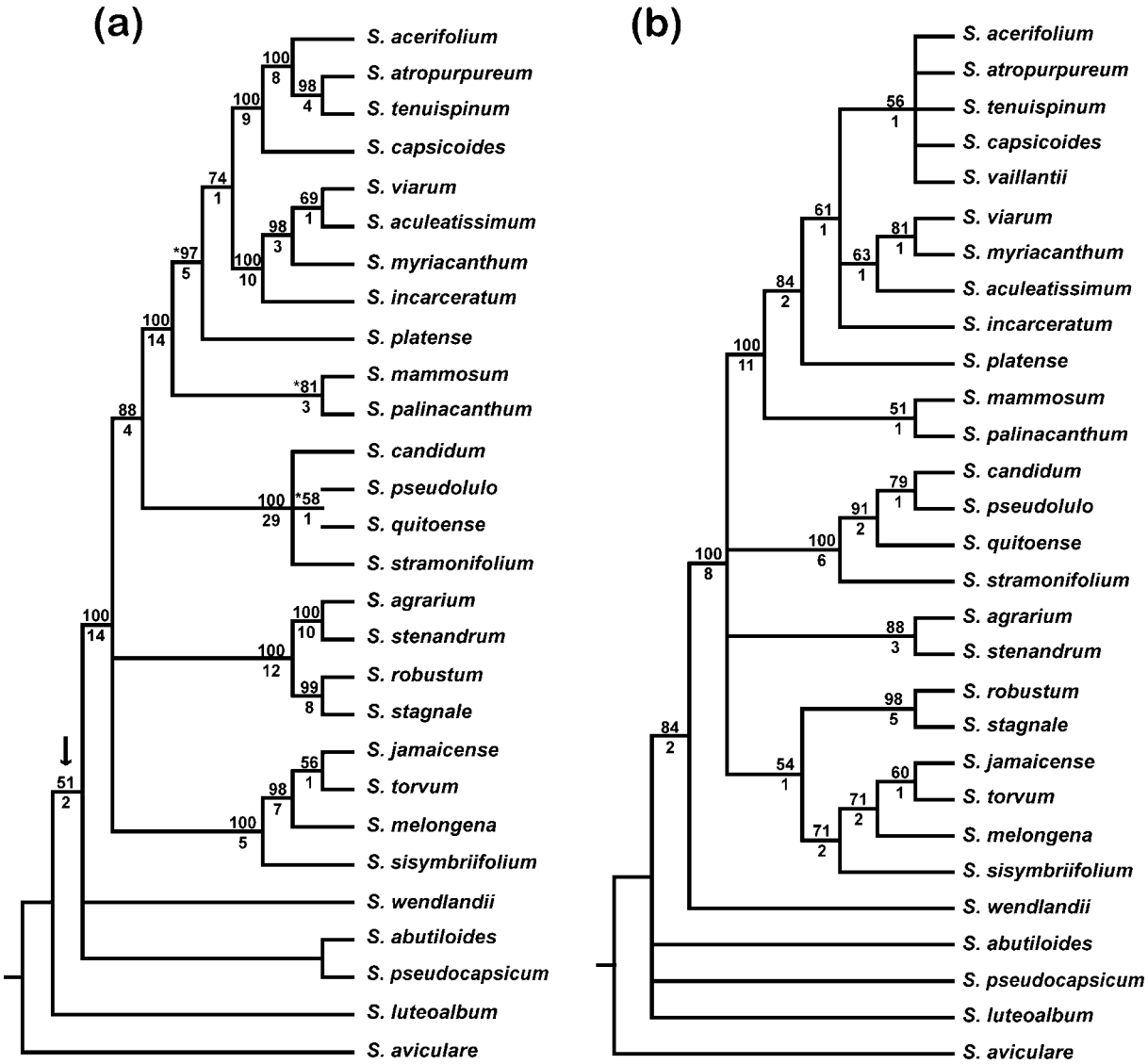

Fig. 3. The strict consensus of most-parsimonious trees (MPTs) inferred from the combined nuclear data sets and the combined chloroplast data sets. Bootstrap values ≥50% are shown above the branches, decay indices below. (a) Strict consensus of nine MPTs inferred from the 28 taxon (*S. vaillantii* excluded due to lack of data) nuclear ITS and *waxy* data sets (tree length = 996; consistency index = 0.74; retention index = 0.74; rescaled consistency index = 0.55). Asterisks indicate those nodes that collapse in the strict consensus topology inferred from the *waxy* data alone; the arrow indicates a node that has much higher support in the *waxy*-only topology (bootstrap values = 71 vs. 51; decay indices = 3 vs. 2). (b) Strict consensus of 210 MPTs inferred from the 29 taxon cp *trnT-trnF* and *trnS-trnG* data sets (tree length = 466; consistency index = 0.87; retention index = 0.86; rescaled consistency index = 0.75).

mated by log-likelihood (RELL) optimization was used, with 1000 bootstrap replicates.

RESULTS

Nuclear data sets—The ITS sequences for 28 taxa ranged in length from 614 to 652 base pairs (bp), with an aligned length of 676 characters, including ITS1, the 5.8S rRNA gene, and ITS2. Of these 676 characters, 113 were parsimony informative (PI) across all 28 taxa, and phylogenetic analysis yielded 60 MPTs of 451 steps. The ITS data show support for a number of nodes. However, the topology (not shown) does not differ from that of the *waxy*-only analysis and the ITS + *waxy* analysis (Fig. 3a), except that *waxy* provides a higher level of resolution. This is not surprising; although the ITS data set has the highest percentage of PI sites, it also has the lowest consistency index (CI) and retention index (RI) of all four data sets, suggesting that there is significant homoplasy in the data, probably as a result of multiple hits (Table 2).

Waxy sequences for 27 taxa ranged in length from 1785 to 1815 bp, with an aligned length of 1831 characters, from the 5′ region of exon 2 through the 3′ end of exon 10 including eight introns (Fig. 2). Of these characters, 139 were parsimony-informative, and phylogenetic analysis yielded 54 MPTs of 533 steps. The topology inferred from the *waxy* data is well resolved and similar to the phylogeny inferred from *waxy* + ITS combined (Fig. 3a); the main difference is that the *waxy*-only analysis strongly supported *S. jamaicense* + *S. torvum* + *S. melongena* + *S. sisymbriifolium* as sister to sect. *Lasiocarpa* + all species in sect. *Acanthophora* excluding *S. stenandrum* and *S. agrarium* (BS = 99; DI = 4).

Results of an ILD test comparing the two nuclear regions, ITS and *waxy,* suggest that the data sets are not congruent ($P < 0.01$). As mentioned above, the topologies inferred from these two regions when analyzed separately do not differ except at the level of resolution. Thus, it appears that the significant incongruence may be due to a large disparity in the size of the partitions (ITS and *waxy*; see Table 2), as well as a difference in the substitution rates between the coding *waxy* region and the noncoding, fast-evolving ITS region, resulting in much higher levels of homoplasy in the ITS data (see also Dolphin et al., 2000; Barker and Lutzoni, 2002; Dowton and Austin, 2002).

The ITS and *waxy* data sets combined (Fig. 3a) show strong support for a clade composed of all species in sect. *Acanthophora* excluding *S. stenandrum* and *S. agrarium* (BS = 100; DI = 14). *Solanum stenandrum* + *S. agrarium* are sister taxa (BS = 100; DI = 10) and are strongly supported as outside of the rest of sect. *Acanthophora*. In the monophyletic sect. *Acanthophora* sensu stricto (s.s.; i.e., excluding *S. stenandrum* and *S. agrarium*), *S. mammosum* + *S. palinacanthum* have moderate support as sister taxa (BS = 81; DI = 3), and together they are sister to all other species in the section (BS = 97; DI = 5). In the remainder of sect. *Acanthophora* s.s., *S. platense* has limited support as sister to all of the other taxa (BS = 74; DI = 1). Among these taxa there are two well-supported lineages; one includes *S. incarceratum* + *S. viarum* + *S. myriacanthum* + *S. aculeatissimum* (BS = 100; DI = 10), with *S. incarceratum* sister to the other three species (BS = 98; DI = 3). In the second clade (BS = 100; DI = 9), *S. capsicoides* is sister to *S. acerifolium* + *S. atropurpureum* + *S. tenuispinum* (BS = 100; DI = 8), with *S. acerifolium* sister to *S. atropurpureum* + *S. tenuispinum* (BS = 98; DI = 4).

Chloroplast data sets—The *trnT-trnF* sequences across 27 taxa ranged in length from 1745 to 2046 bp, with an aligned length of 2397 characters. This considerable range in length reflects the high number of indels in this data set. Of these characters, 82 were parsimony-informative, and phylogenetic analysis yielded 693 MPTs of 326 steps. The sequences of the *trnS-trnG* spacer region across 29 taxa ranged in length from 656 to 708 bp, with an aligned length of 819 characters. Of these characters, 36 were parsimony-informative, and phylogenetic analysis yielded 6282 MPTs of 138 steps. These two chloroplast regions appear to have comparable amounts of information, with similarly high consistency indices and percentage of PI characters (Table 2). The data sets also have congruent phylogenetic signal. Yet, as the *trnT-trnF* data set has many more characters, it is not surprising that the strict consensus topology inferred from this data set is much better resolved than that from the *trnS-trnG* data set (trees not shown).

An ILD test of the chloroplast data sets confirmed that they are highly congruent ($P = 0.85$). Overall the phylogeny inferred from these two data sets combined is quite similar to that inferred from the combined nuclear data (Fig. 3). The exception is the placement of *S. robustum* + *S. stagnale* as sister to *S. agrarium* + *S. stenandrum* (BS = 100; DI = 12) in the combined nuclear topology, whereas in the combined cp topology *S. robustum* + *S. stagnale* are weakly supported in a clade with *S. sisymbriifolium* + *S. melongena* + *S. torvum* + *S. jamaicense*. The *trnS-trnG* data set is also the only one that includes data for *S. vaillantii*. From the combined cp analysis *S. vaillantii* appears to belong in the clade including *S. acerifolium*, *S. atropurpureum*, *S. capsicoides*, and *S. tenuispinum* (BS = 56; DI = 1); however, more sequence data are needed to confirm this placement.

All data sets combined—Despite the apparent incongruence of the ITS and *waxy* data sets, results of an ILD test comparing all four 27 taxa data sets simultaneously suggest that the data sets are highly congruent ($P = 0.17$), reflecting the similar topologies of the phylogenies inferred from each individual data set. To verify this result, all pairwise ILD tests were also conducted, and the only significant incongruence occurred between the two nuclear data sets. The combined data set included 5723 characters for 27 taxa (*S. agrarium* and *S. vaillantii* were excluded), of which 358 were parsimony-informative. The phylogenetic analysis resulted in eight MPTs of 1452 steps. There is very strong support for most nodes in the strict consensus topology (Fig. 4). Subgenus *Leptostemonum* is well supported as monophyletic (BS = 100; DI = 25), as is a sister relationship between sects. *Acanthophora* s.s. and *Lasiocarpa* (BS = 93; DI = 4).

As noted above, sect. *Acanthophora* as currently circumscribed does not appear to be monophyletic. *Solanum stenandrum* (and *S. agrarium*, due to its confident placement as sister to *S. stenandrum* in Fig. 3) is strongly supported as outside of the *Acanthophora* + *Lasiocarpa* lineage. Within the monophyletic sect. *Acanthophora* s.s. (i.e., excluding *S. stenandrum* and *S. agrarium*) relationships are as discussed for the combined nuclear analysis, but with increased nodal support.

Maximum likelihood—Maximum likelihood (ML) analysis of the four region combined data set was conducted with parameters estimated using Modeltest. This procedure indicated that the GTR + I + G model best fit the data. The ML model parameters included nucleotide frequencies of A = 0.3039, C = 0.192, G = 0.1902, and T = 0.3139; a substitution rate matrix of A to C: 1.0083, A to G: 1.611, A to T: 0.4475, C to G: 1.0864, C to T: 2.3434, and G to T: 1; a proportion of invariant sites = 0.4971; and a gamma rate distribution at variable sites with shape (alpha) = 0.8423. Using this model and all eight MPTs from the combined data set as the starting trees, this analysis yielded one tree with $-\ln = 17014.40718$. The ML topology (not shown) is identical to the topology inferred assuming parsimony (Fig. 4), although the single ML tree (as it is not a consensus tree) resolved the polytomies present in the most-parsimonious strict consensus tree.

DISCUSSION

Comparative utility of the four gene regions—All four data sets, including two regions from the chloroplast genome and two from the nuclear genome, resulted in similar topologies. However, in terms of phylogenetic utility, the nuclear GBSSI (*waxy*) gene was the most useful, having both a high percentage of PI characters as well as high consistency and retention indices, suggesting a low level of homoplasy (Table 2). It is likely that the high information content of this region is due to the mix of both noncoding introns and coding exons (Fig. 2). Not surprisingly, there were only 54 PI characters (5%) of 1089 total exon characters; in contrast, there were 85 PI characters (11%) of 742 total intron characters. There is a considerable amount of variation in the number of PI characters across the various exons and introns (Fig. 5), with the result that the 3′ end of the gene appears somewhat more phyloge-

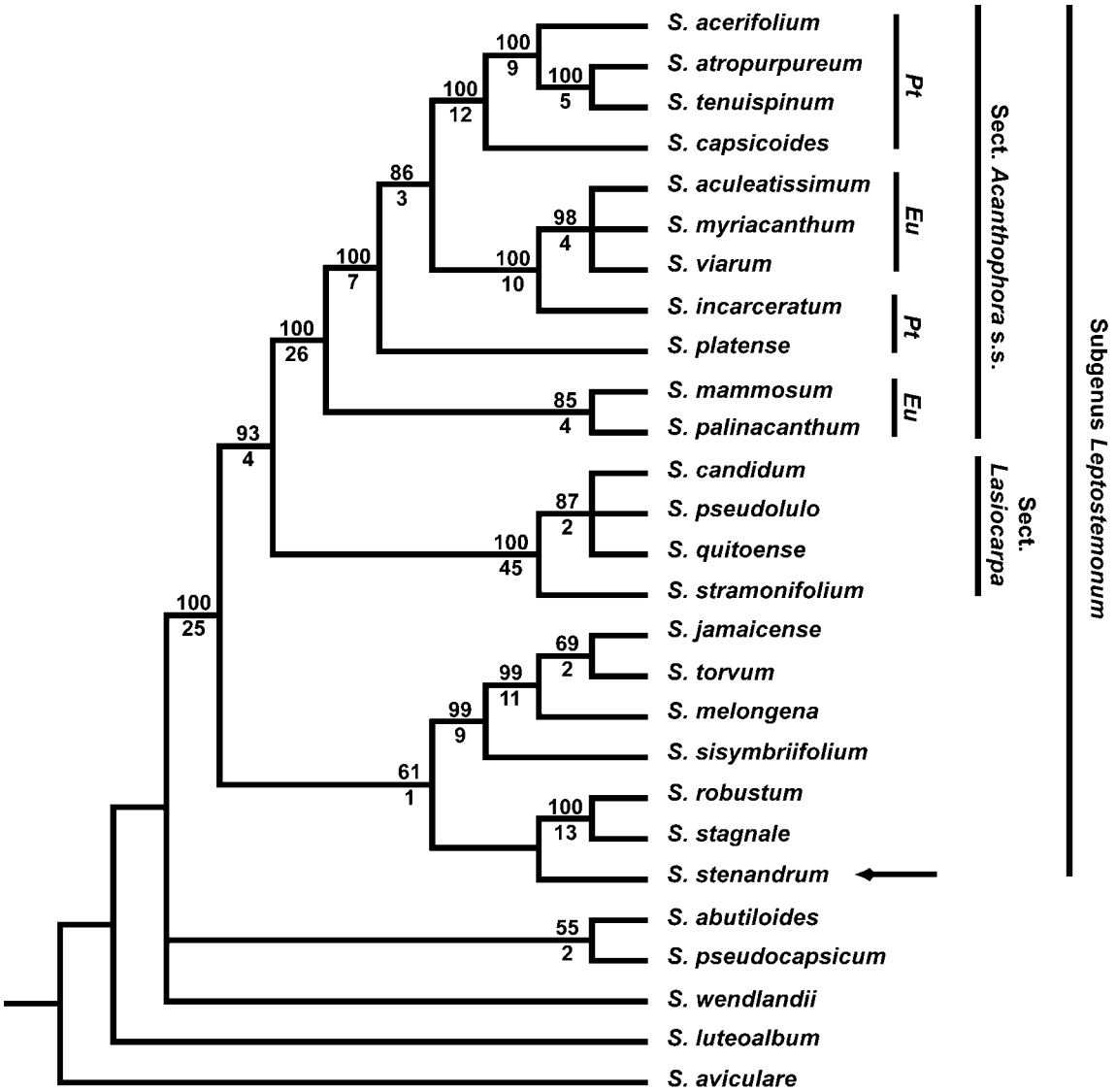

Fig. 4. The strict consensus of the eight most-parsimonious trees inferred from all four data sets combined (ITS, *waxy*, *trnT-trnF*, and *trnS-trnG*) including the 27 taxa for which data from all gene regions were available (tree length = 1452; consistency index = 0.78; retention index = 0.77; rescaled consistency index = 0.60). Bootstrap values ≥50% are shown above the branches, decay indices below. Pt = Pterosperma group; Eu = Euleptostemonum group, corresponding to subsections circumscribed by Nee (1979a; Table 1). Arrow indicates the location of *Solanum stenandrum* (and *S. agrarium*, see Fig. 3) outside of the rest of section *Acanthophora*.

netically informative than the 5′ end of the gene. The nuclear ITS data set had a higher percentage of PI characters than *waxy*; however, its high level of homoplasy makes ITS less useful than *waxy* for resolving relationships. The two chloroplast spacer regions were also phylogenetically informative, but as is typical of many chloroplast regions, they are not as rapidly evolving and thus have a lower percentage of PI characters than either *waxy* or ITS. However, their utility is enhanced by their low level of homoplasy, and combined with the other data sets, they contributed to a robust phylogenetic hypothesis.

Higher level relationships—Sections *Acanthophora* s.s. and *Lasiocarpa* are well supported as sister taxa, with the main differences being the predominance of simple hairs on the stems and upper leaf surfaces and glabrous fruits at maturity in sect. *Acanthophora* vs. the stellate hairs and pubescent fruits found in sect. *Lasiocarpa* (Nee, 1979a). It is possible that taxa in the *Solanum wacketii* species group are more closely related to sect. *Acanthophora* than is sect. *Lasiocarpa*; however, the lack of available material for species in the *S. wacketii* group precludes examination of its phylogenetic placement.

The taxon sampling for this analysis does not permit the sister group of sects. *Acanthophora* s.s. + *Lasiocarpa* to be determined with certainty, although analysis of a much larger data set (R. A. Levin, N. R. Myers, and L. Bohs, unpublished manuscript) suggests that this clade is not basal within subgenus *Leptostemonum* (contra Bohs, in press). The simple hairs characteristic of sect. *Acanthophora* are likely derived by reduction from stellate hairs (Nee, 1979a). Reduction of stellae into simple hairs may have occurred several times independently in subg. *Leptostemonum*, for example, on the branches

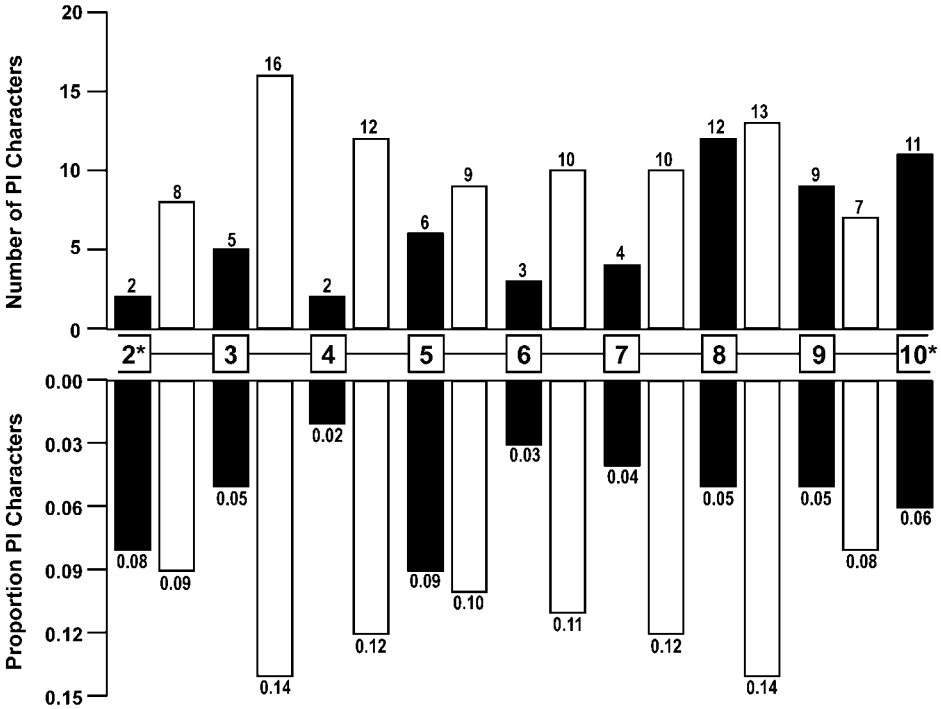

Fig. 5. The total number of parsimony-informative (PI) characters and the proportion of PI characters per exon (solid bars) and intron (open bars) in the nuclear GBSSI (*waxy*) gene across the taxa in this study. Asterisks indicate that there are only partial data for the first and last exons sequenced (i.e., the 3′ region of exon 2 and the 5′ region of exon 10). Note that the lengths of the exons and introns are not shown to scale (see Fig. 2).

leading to sect. *Acanthophora* s.s., in *S. pectinatum* (sect. *Lasiocarpa*), and in *S. stenandrum* + *S. agrarium*. Further analyses in progress will help elucidate relationships within subg. *Leptostemonum* (R. A. Levin, N. R. Myers, and L. Bohs, unpublished manuscript), and developmental studies of trichomes within the spiny solanums may clarify the evolutionary origin(s) of simple hairs.

Monophyly of section Acanthophora—All four data sets strongly suggest that sect. *Acanthophora* is not monophyletic as currently circumscribed (Figs. 3–4). Clearly, *S. stenandrum* and *S. agrarium* (both members of the Euleptostemonum group) belong outside of sect. *Acanthophora* and outside of the lineage composed of all species in sects. *Acanthophora* s.s. + *Lasiocarpa*. Constraining sect. *Acanthophora* s.l. to be monophyletic requires 25 more steps, and these constrained topologies have significantly lower likelihood values (one-tailed S-H test; $P < 0.001$ across all constrained topologies).

From the limited molecular data provided by both the ITS and *trnS-trnG* regions (these were the gene regions for which we were able to obtain sequence data for *S. agrarium*), it appears that *S. stenandrum* and *S. agrarium* are sister taxa. Further, both share many characters, including a thickened margin around the seed, with the distinguishing features being smaller fruits and fewer seeds in *S. stenandrum* (Nee, 1979a). Based on morphology, the relationship of these two taxa to others in the Euleptostemonum group was equivocal (Nee, 1979a). Thus, perhaps it is not surprising that these two species do not even belong within sect. *Acanthophora*. While it is apparent from the present study that *S. stenandrum* + *S. agrarium* belong outside of sects. *Acanthophora* s.s. + *Lasiocarpa*, their closest relatives may not be *S. robustum* + *S. stagnale* as suggested by Figs. 3–4. Further taxon sampling within subgenus *Leptostemonum* is needed to fully understand the closest relatives of *S. agrarium* and *S. stenandrum* (R. A. Levin, N. R. Myers, and L. Bohs, unpublished manuscript). Although Nee (1979a) segregated *S. tenuissimum*, an unsampled member of sect. *Acanthophora*, in a separate group due to its diminutive stature, small leaves and flowers, and small few-seeded fruits, he suggested that it may be related to *S. stenandrum*. Both species occur in cerrado vegetation in the Planalto of northeastern and central Brazil and have similar pubescence and small, relatively few-seeded fruits. However, fruits of *S. stenandrum* contain about 30–80 seeds, whereas those of *S. tenuissimum* contain only 1–3 seeds.

Relationships within section Acanthophora—Within sect. *Acanthophora*, the groups outlined by Nee (1979a) are not monophyletic (Table 1, Fig. 4). *Solanum palinacanthum* + *S. mammosum* are clearly sister to the rest of sect. *Acanthophora* s.s., being united by the presence of purple corollas and large fruits with a spongy mesocarp (Nee, 1979a). Within the strongly supported clade including the remaining taxa, a reduced Euleptostemonum group (i.e., excluding *S. palinacanthum* + *S. mammosum* as well as *S. stenandrum* + *S. agrarium*) appears nested within the Pterosperma group. *Solanum aculeatissimum* + *S. myriacanthum* + *S. viarum* are thought to be very closely related based on morphology, and Nee (1979a) speculates that the three species may form a hybrid complex, evidence that is concordant with the strong monophyly of these three species based on molecular data (Fig. 4).

The other well-supported lineage includes *S. capsicoides* + *S. acerifolium* + *S. atropurpureum* + *S. tenuispinum*. The results from the combined chloroplast analysis (Fig. 3b), in which *S. vaillantii* was included, suggest that this species also belongs within this lineage. According to Nee (1979a), these

five species share many morphological characters, including the presence of winged seeds. He suggested that although *S. platense* also has winged seeds, it is not that closely related to *S. capsicoides*, *S. atropurpureum*, *S. tenuispinum*, and *S. acerifolium*. The molecular results support this hypothesis of relationships and also show that *S. incarceratum*, another winged seed species, is not a member of the *S. capsicoides* + *S. acerifolium* + *S. atropurpureum* + *S. tenuispinum* clade. The results presented here suggest that winged seeds may have evolved along the branch leading to all species of sect. *Acanthophora* s.s. except *S. palinacanthum* + *S. mammosum*, with a loss of the winged morphology occurring in the common ancestor of *S. aculeatissimum* + *S. myriacanthum* + *S. viarum*. Micromorphological and developmental studies of wingless seeds from the two clades that were formerly united in the Euleptostemonum group may provide additional evidence for the reduction or loss of the seed wing within sect. *Acanthophora*.

A rough correlation exists between species with relatively large (>2 cm diam.), presumably mammal-dispersed fruits and the absence of a seed wing. These characters occur together in *S. mammosum*, *S. palinacanthum*, *S. viarum*, *S. myriacanthum*, and *S. aculeatissimum*. Conversely, *S. incarceratum*, *S. tenuispinum*, *S. atropurpureum*, and *S. acerifolium* have small, presumably bird-dispersed fruits and winged seeds. However, the large-fruited *S. capsicoides* and *S. platense* also possess winged seeds. Both of these species are adapted to sandy coastal habitats and both reportedly have a shaker-type mechanism of seed dispersal (Nee, 1979a). Further, according to Nee (1979a), *S. capsicoides* seeds can float. However, the functional significance of winged seeds in bird-dispersed fruits is puzzling. Experiments with *S. acerifolium* and *S. atropurpureum* showed that seeds of these species do not float, but *S. atropurpureum* seeds are sticky at maturity and may adhere to birds' feet or feathers (Nee, 1979a). More observations on fruit morphology and dispersal agents are needed to understand the correlation, if any, between fruit and seed characters and dispersers in species of sect. *Acanthophora*.

Fruit glycoalkaloids—Cipollini and Levey (1997) suggested a correlation between seed-dispersal syndromes and glycoalkaloid (GA) levels in fruits, with low GA amounts in bird-dispersed fruits, intermediate GA levels in bat-dispersed fruits, and high GA content in fruits dispersed by non-flying mammals. Cipollini et al. (2002) tested this idea by examining total glycoalkaloid (TGA) content in ripe fruits of five species in sect. *Acanthophora*. Preliminary results contradict the proposed pattern, with *S. capsicoides* and *S. mammosum*, both putatively dispersed by non-flying mammals (i.e., large-fruited species), having widely different levels of fruit GAs (Cipollini et al., 2002). *Solanum mammosum* fruits contained almost 50 times more TGA than those of *S. capsicoides*, and TGA levels in *S. mammosum* fruits were higher than those of any other *Solanum* species investigated. The closely related *Solanum viarum* and *S. myriacanthum* are intermediate in fruit size and have very similar fruit morphologies, but they differ in fruit GA levels, with *S. myriacanthum* having a much higher TGA content than *S. viarum*. *Solanum acerifolium*, with small and presumably bird-dispersed fruits, has low fruit GA content, but not as low as its close relative, the large-fruited *S. capsicoides*. Thus, it appears that neither phylogenetic constraints nor fruit morphological traits (and, by implication, dispersal syndromes) seem to be correlated with fruit TGA levels in sect. *Acanthophora*. Further investigations are warranted to document the actual fruit dispersers of these taxa, to examine in more detail the infraspecific variation in fruit GA content, and to sample all the species of sect. *Acanthophora* for fruit morphological and chemical traits.

Conclusions—*Solanum* sect. *Acanthophora* is clearly not monophyletic as currently circumscribed. Rather, a more strict definition of the section is warranted, with the exclusion of *Solanum agrarium* and *S. stenandrum*. Analyses with much greater taxon sampling within subgenus *Leptostemonum* will help clarify the true taxonomic affinities of these two species (R. A. Levin, N. R. Myers, and L. Bohs, unpublished manuscript). In comparison to the commonly used nuclear ITS region and two chloroplast spacer regions (*trnT-trnF* and *trnS-trnG*), the nuclear *waxy* gene has both the most parsimony-informative sites as well as low homoplasy. Although the nrITS region has the highest proportion of parsimony-informative characters, its high level of homoplasy makes it less desirable for phylogenetic inference among these taxa. The *waxy* gene is a valuable addition to the tools of plant molecular systematics in inferring robust hypotheses of evolutionary relationships and may be especially useful where ITS appears to have evolved too rapidly for the question at hand.

Solanum sect. *Acanthophora* is a promising group for the study of fruit and seed morphology, fruit chemistry, and dispersal agents in a phylogenetic context. Additional studies, especially field observations, may reveal the functional significance of the morphological and biochemical diversity seen among the species of this section and shed light on the ecological interactions between members of sect. *Acanthophora* and their fruit and seed dispersers.

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