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A Chloroplast DNA Phylogeny of *Solanum* Section *Lasiocarpa*

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ABSTRACT. *Solanum* section *Lasiocarpa* includes about a dozen species with a center of diversity in the New World tropics. *Solanum lasiocarpum* and *S. repandum* (sometimes considered to be conspecific as *S. ferox*) have an Old World distribution in Asia and the Pacific Islands. Several species in this section produce edible fruits, and two, the lulo or naranjilla (*S. quitoense*) and the cocona (*S. sessiliflorum*) are cultivated commercially. Phylogenetic relationships in *Solanum* section *Lasiocarpa* were investigated using sequence data from the chloroplast *trnT-trnL* spacer, the *trnL-trnF* spacer, and the *trnL* gene, including the *trnL* intron. Sampling included 24 accessions from section *Lasiocarpa* and 14 accesssions of other *Solanum* species as outgroups. All species considered to belong to section *Lasiocarpa* by previous authors were examined with the exception of the recently described *S. atheniae. Solanum robustum* and *S. stagnale*, sometimes considered to belong to section *Lasiocarpa*, are excluded from the group on the basis of the *trn* data. The remaining species in the section form a monophyletic group, with three well-supported clades within it: *S. hirtum*, *S. pectinatum-sessiliflorum-stramonifolium*, and the remainder of the species in the section. Sequences of *S. lasiocarpum* and *S. repandum* are extremely similar, and these two Asian taxa cluster with the New World *S. candidum* and *S. pseudolulo* on the *trn* trees.

Solanum section *Lasiocarpa* (Dunal) D'Arcy comprises approximately a dozen species of perennial shrubs or small trees with a center of distribution in northwestern South America. Morphological characters that define the section include difoliate sympodial units, large repand leaves, unbranched inflorescences, stellate corollas, and fruits covered with stellate hairs with reduced lateral rays (Whalen et al. 1981). The section was monographed by Whalen et al. (1981), who recognized 13 species. Eleven are native to the northern Andes of Venezuela, Colombia, Ecuador, and Peru, and three have ranges that extend into Central America (*S. candidum*, *S. hirtum*) or northeastern South America through the Guianas into northern Brazil (*S. stramonifolium*). Several species in the section produce edible fruits and two, *S. quitoense* (the lulo or naranjilla) and *S. sessiliflorum* (the cocona) are economically important fruit crops in Latin America (Heiser 1969, 1985a). *Solanum quitoense* has been introduced to Panama, Costa Rica, and Guatemala and is now naturalized in Central America. *Solanum lasiocarpum* and *S. repandum* are found in Asia and the Pacific Islands. Although treated as separate taxa by Whalen et al. (1981), Heiser (1996) considered them conspecific under the name *S. ferox*.

Dunal (1852), Morton (1976), and Hunziker (2001) included the South American *S. robustum* in section *Lasiocarpa*, but Whalen et al. (1981) excluded it from the section due to differences in branching pattern, leaf shape, and fruit trichomes. Subsequent to Whalen et al.'s (1981) treatment, *S. stagnale* was removed from the section and placed in the *S. polytrichum* group within *Solanum* subgenus *Leptostemonum* (Dunal) Bitter (Whalen 1984; Child 1998; Nee 1999). Symon (1985) described *S. atheniae* from New Guinea and postulated that it belonged to section *Lasiocarpa*.

Solanum section *Lasiocarpa* belongs to the spiny subgroup of the genus *Solanum*, usually recognized as *Solanum* subgenus *Leptostemonum*. Previous authors such as Dunal (1852), Seithe (1962), Danert (1970), D'Arcy (1972), and Whalen (1984) have regarded subgenus *Leptostemonum* as a natural group based on the shared presence in most species of spines, stellate hairs, and tapered anthers. Molecular phylogenetic studies based on chloroplast DNA restriction sites (Olmstead and Palmer, 1997) and nuclear and chloroplast sequence data (Bohs and Olmstead 1997, 1999, 2001; Bohs, in press) indicate that *Solanum* species that bear spines as well as stellate hairs comprise a monophyletic group, termed the Leptostemonum clade by Bohs (in press). *Solanum wendlandii*, a representative of *Solanum* section *Aculeigerum* Seithe, falls outside the clade comprised of the other spiny *Solanum* taxa (Bohs and Olmstead 1997, 1999, 2001; Bohs, in press). *Solanum* section *Aculeigerum* includes six species that bear spines but lack stellate hairs. In this paper, *Solanum* subgenus *Leptostemonum* is used in the traditional sense to refer to all taxa of the genus that bear spines. The term Leptostemonum clade is used in accordance with Bohs (in press) to refer to the monophyletic group of spiny *Solanum* taxa exclusive of *Solanum* section *Aculeigerum*.

Molecular studies based on chloroplast DNA restriction sites and chloroplast *ndhF* sequence data using a broad range of sampling from *Solanum* indicate that section *Lasiocarpa* may be a relatively basal lineage within the Leptostemonum clade and that it may be sister to *Solanum* section *Acanthophora* Dunal (Olmstead and Palmer 1997; Bohs, in press). Whereas these broad scale studies sampled only one to two species from the section, species-level relationships in section *Lasiocarpa* have been the subject of numerous investigations using morphological data, crossing studies, isozyme electrophoresis, karyotype analyses, and cpDNA restriction

TABLE 1. Sources of *Solanum* DNA accessions used in this study. Seeds, leaves, or DNA extracts provided by [1] L. Bohs, University of Utah, Salt Lake City, UT. [2] R. G. Olmstead, University of Washington, Seattle, WA. [3] A. Bruneau, McGill University, Montreal, Canada. [4] C. B. Heiser, Jr., Indiana University, Bloomington, IN. [5] J. Miller, Amherst College, Amherst, MA. [a] For further collection and voucher data see Appendix in Whalen et al. (1981). BIRM samples bear the seed accession number of the University of Birmingham Solanaceae collection. Nijmegen accession numbers refer to the Solanaceae collection at the University of Nijmegen, The Netherlands.

Solanum* section *Lasiocarpa*: *S. candidum Lindl.[3]—*Stoutamire s.n.* (IND) from *Heiser S249*[a], Mexico: Veracruz (AY266250). ***S. candidum*** Lindl.[1]—*Bohs 2898* (UT), Costa Rica: La Cangreja (AY266237). ***S. felinum*** Whalen[4]—*Benitez de Rojas 8915* (IND), Venezuela: Colonia Tovar (AY266252). ***S. hirtum*** Vahl[3]—*Whalen 730* (QCA), Ecuador (AY266254). ***S. hirtum*** Vahl[3]—*Jones s.n.* (IND) from *Heiser S404*[a], Costa Rica: Guanacaste (AY266253). ***S. hyporhodium*** A. Braun & Bouché[3]—*Whalen 717* (BH), Venezuela: Sucre (AY266238). ***S. hyporhodium*** A. Braun & Bouché[4]—*Carreno Espinosa 8214* (IND), Venezuela: Sucre (AY266255). ***S. lasiocarpum*** Dunal[4]—*Ansyar 9605* (IND), Indonesia: Pandang (AY266256). ***S. pectinatum*** Dunal[4]—*Peeke 8512* (IND), Ecuador: Limoncocha (AY266227). ***S. pectinatum*** Dunal[1]—*Bohs 2899* (UT), Bolivia: Santa Cruz (AY266230). ***S. pseudolulo*** Heiser[3]—*Plowman et al. 4276* (GH)[a], Colombia: Meta, Sierra de la Macarena (AY266258). ***S. pseudolulo*** Heiser[5]—Bohs DNA extract 995, Nijmegen #824750021 (AY266242). ***S. quitoense*** Lam.[1]—*Bohs 2873* (UT), Costa Rica (AY266228). ***S. quitoense*** Lam.[4]—*Heiser s.n.* Bohs DNA extract 996, Ecuador: Quito market (AY266243). ***S. repandum*** G. Forst.[3]—*Heiser 8215* (IND), Fiji (AY266229). ***S. repandum*** G. Forst.[4]—*Ashley 8627* (IND), Solomon Islands: Malaita (AY266234). ***S. sessiliflorum*** Dunal[3]—*Dickson 458* (BH) from *Whalen 859* (HUT), Peru (AY266261). ***S. sessiliflorum*** Dunal var. ***sessiliflorum***[4]—*Heiser 8255* (IND), Ecuador: Yanzatza (AY266260). ***S. stramonifolium*** Jacq. var. ***inerme*** (Dunal) Whalen[4]—*Pickersgill 154* (IND), Peru: Iquitos (AY266244). ***S. stramonifolium*** Jacq. var. ***inerme*** (Dunal) Whalen[3]—*Whalen & Salick 860* (BH), Peru: Pasco, Iscozacin (AY266263). ***S. vestissimum*** Dunal[3]—*Dickson 456* (BH) from *Plowman 13431* (F), Venezuela (AY266264). ***S. vestissimum*** Dunal[4]—*Movilla s.n.* (IND) from *Heiser S432*[a], Colombia: Santa Marta (AY266247).

Outgroups: ***S. abutiloides*** (Griseb.) Bitter & Lillo[2]—*RGO S-73* (WTU), BIRM S.0655 (AY266236). ***S. acerifolium*** Dunal[1]—*Bohs 2714* (UT), Costa Rica (AY266249). ***S. capsicoides*** All.[1]—*Bohs 2451* (UT), Peru (AY266251). ***S. dulcamara*** L.[2]—no voucher, USA (AY266231). ***S. jamaicense*** Mill.[2]—*RGO S-85* (WTU), BIRM S.1209 (AY266239). ***S. luteoalbum*** Pers.[1]—*Bohs 2337* (UT), BIRM S.0042 (AY266257). ***S. mammosum*** L.[2]—*RGO S-89* (WTU), BIRM S.0983 (AY266232). ***S. melongena*** L.[2]—*RGO S-91* (WTU), BIRM S.0657 (AY266240). ***S. palinacanthum*** Dunal[1]—*Bohs 3151* (UT), Bolivia (AY266233). ***S. pseudocapsicum*** L.[2]—no voucher, BIRM S.0870 (AY266241). ***S. robustum*** Wendl.[4]—*Bohs 3084* (UT), Argentina: Corrientes, Perichón (AY266259). ***S. sisymbriifolium*** Lam.[1]—*Bohs 2533* (UT), Argentina (AY266235). ***S. stagnale*** Moric.[4]—*Carvalho 3213* (IND), Brazil: Bahia, Valença (AY266262). ***S. tenuispinum*** Rusby[1]—*Bohs 2475* (UT), Bolivia (AY266245). ***S. torvum*** Sw.[1]—*RGO S-101* (WTU), BIRM S.0839 (AY266246). ***S. wendlandii*** Hook. f.[1]—no voucher, BIRM S.0488 (AY266248).

site data (Heiser 1972, 1985b, 1987, 1989; Whalen et al. 1981; Whalen and Caruso 1983; Bernardello et al. 1994; Bruneau et al. 1995). Many of these studies were aimed at examining the evolutionary history of the Asian disjuncts and the origin and evolution of *S. quitoense*. Despite the accumulation of an impressive amount of data, a consensus has not been reached regarding the phylogenetic relationships of the taxa of this group due to conflicting topologies from different data sets and to low resolution in some parts of the trees. Evidence suggests that the Asian species *S. repandum* and *S. lasiocarpum* are sister taxa (Heiser 1986, 1987; Bernardello et al. 1994; Bruneau et al. 1995) or even conspecific (as *S. ferox*; Heiser 1996), but the closest relatives of this clade are debated. The inclusion of *S. stagnale*, *S. robustum*, and *S. atheniae* in section *Lasiocarpa* has not been critically examined and the data have been inconclusive with respect to the wild relatives of the putative domesticates *S. quitoense* and *S. sessiliflorum*.

The present study examines species-level phylogenetic relationships in *Solanum* section *Lasiocarpa* using chloroplast *trn* sequence data. These data shed light on the circumscription of the section, the relationships of the Asian taxa, and the wild relatives of the lulo and cocona, and demonstrate the utility of *trn* sequence data for examining species-level phylogeny within *Solanum*.

MATERIALS AND METHODS

All species placed in section *Lasiocarpa* by Whalen et al. (1981) were sampled, including *S. stagnale* and *S. robustum*. *Solanum atheniae* is known only from the type (Symon 1985) and no material was available for sampling. In most cases, two accessions were sampled from each species of section *Lasiocarpa*. Outgroup taxa included ten species from *Solanum* subgenus *Leptostemonum* and four species representing taxa from various non-spiny *Solanum* clades. Outgroups were chosen to represent a variety of diverse *Solanum* clades based on previous molecular studies. In addition, sampling included five representatives from *Solanum* section *Acanthophora*, which was identified as the sister group to section *Lasiocarpa* in previous analyses based on chloroplast DNA data (Olmstead and Palmer 1997; Bohs, in press). Collection, voucher, and GenBank information is given in Table 1.

DNA was extracted from fresh or silica dried leaf material using protocols described in Bohs and Olmstead (1997, 2001) and Bohs (in press). Amplification of the entire *trnT* (UGU)—*trnF* (GAA) region used primers a and f of Taberlet et al. (1991) in 25 µl reactions as described in Bohs and Olmstead (2001) with a PCR program of 92° C for 7 min followed by 30 cycles of 92° C for 1 min, 45° C for 1 min, 72° C for 5 min, and a single cycle of 72° C for 7 min. PCR products were cleaned using QiaQuick spin columns (Qiagen, Inc., Valencia, CA) and sequenced on an ABI automated sequencer using primers a through f of Taberlet et al. (1991).

Sequence data were edited and contigs constructed using Sequencher (Gene Codes Corp.) and sequences were aligned by eye using Se-Al (Rambaut 1996). Indel alignments took into account the mechanisms and patterns of evolution in non-coding sequences outlined in Kelchner (2000). All sequences were submitted to GenBank (Table 1) and the data sets and representative trees are

deposited in TreeBASE [accession numbers S907 (study) and M1490 (matrix)].

The *trn* region sampled here includes two coding regions (*trnL* 5′ and 3′ exons), two intergenic spacers (*trnT–trnL* and *trnL–trnF* spacers), and the *trnL* intron (for diagrams and sequences of this region in tobacco, see Yamada et al. 1986). To explore the informativeness of each of these regions in the context of *Lasiocarpa* phylogeny, each of the non-coding regions was analyzed separately using parsimony and the results were compared with those from the complete data sets.

To explore the effects of indels and indel coding on the phylogenetic results, several analyses were performed on the aligned data set. The first used the complete aligned nucleotide sequence data set, with gaps treated as missing data. The second excluded indels from the sequence data matrix. For subsequent analyses, 32 phylogenetically informative gap characters (i.e., those shared by two or more taxa) whose homology could be confidently assessed were coded as separate presence/absence characters according to the simple indel coding scheme of Simmons and Ochoterena (2000). The third analysis used the nucleotide sequence data with indels excluded and the 32 presence/absence gap characters added. The fourth analysis used the complete aligned sequence data, including indel regions, with the addition of the 32 presence/absence gap characters.

Parsimony analyses were conducted with PAUP* 4.0b10 (Swofford 2002) using the heuristic search algorithm with the TBR, MulTrees, and Steepest Descent options, equal weights for all characters and character state changes, and 500 random-order entry replicates. Bootstrap analyses were performed with 500 replicates using the heuristic search option, TBR and MulTrees, Maxtrees set to 1,000, and rearrangements limited to 1,000,000 per replicate.

Sequence data from the ITS region were obtained from a subset of the *Lasiocarpa* species used in the *trn* study using protocols described in Bohs and Olmstead (2001). ITS sequence divergence was extremely low among *Lasiocarpa* taxa and provided little phylogenetic information. These ITS sequences were deposited in GenBank (numbers AY263455—AY263467), but are not analyzed further here.

Results

The total length of the *trn* aligned sequence dataset was 2334 nucleotides, of which 791 represented indels. The total unaligned length of *trn* sequences ranged from 1759 to 2052 bp in species of section *Lasiocarpa* and from 1673 to 1955 bp in the outgroups (Table 2). Lengths of the *trnT-trnL* and *trnL-trnF* intergenic spacers and the components of the *trnL* gene are given in Table 2 for each accession sequenced. Each of these regions provided different numbers of characters for phylogenetic analyses (Table 3).

Of the 2334 characters in the complete aligned sequence data set, 212 were variable and 75 of these were parsimony-informative. Parsimony analysis of this data set found 1907 most parsimonious trees of 263 steps, with a consistency index (CI; excluding uninformative characters) of 0.761 and a retention index (RI) of 0.909 (Fig. 1). In the second analysis, regions with indels were excluded from the aligned sequence data set. Of 1543 total characters, 167 were variable and 57 of these were parsimony-informative. PAUP* found 796 most parsimonious trees of 210 steps, with a CI of 0.747 and an RI of 0.913. The third analysis used the aligned sequence data minus indels with the 32 presence/absence indel characters added. Of the 1575 total characters in this data set, 199 were variable with 89 of these parsimony-informative. This analysis resulted in 3205 trees of 270 steps with a CI of 0.667 and RI of 0.882. The final parsimony analysis used the complete aligned sequence data with the coded indels, resulting in a total of 2366 characters. Of these, 244 were variable and 107 were parsimony-informative. The analysis found 3194 trees of 323 steps with a CI of 0.688 and RI of 0.882.

All four parsimony analyses described above resolved the following clades, which were present in all the strict consensus trees: 1) All the spiny taxa of *Solanum* (i.e., *Solanum* subgenus *Leptostemonum*) with the exception of *S. wendlandii* formed a monophyletic group with 100% bootstrap support in all analyses. *Solanum wendlandii*, an anomalous spiny taxon sometimes placed in subgenus *Leptostemonum*, fell outside the spiny clade. *Solanum wendlandii* was also excluded from the Leptostemonum clade in previous molecular analyses (Bohs and Olmstead 1997, 1999, 2001; Bohs, in press). 2) All species of *Solanum* section *Lasiocarpa* formed a monophyletic group, with 87–93% bootstrap support depending on the analysis. *Solanum robustum* and *S. stagnale*, sometimes put into section *Lasiocarpa*, did not group with the traditional members of the section, but instead emerged as sister taxa within the Leptostemonum clade. 3) The five species of *Solanum* section *Acanthophora* included in this study (*S. acerifolium*, *S. capsicoides*, *S. mammosum*, *S. palinacanthum*, and *S. tenuispinum*) also formed a monophyletic group with 100% bootstrap support. 4) All analyses identified a clade within section *Lasiocarpa* consisting of *S. sessiliflorum*, *S. stramonifolium*, and *S. pectinatum*. Bootstrap support for this group ranged from 93–97% depending on the analysis. 5) Within this latter clade, the two accessions each of *S. sessiliflorum* and *S. stramonifolium* grouped together with 94–98% and 63–98% bootstrap support, respectively.

Other clades were resolved in one or more of the analyses, but either do not appear on the strict consensus trees from the individual analyses or were not resolved in all four analyses. The 50% majority rule consensus trees from Analyses 1 and 2 resolved identical clades within the ingroup (Fig. 2). In addition to the groups described above, these analyses identified the following relationships: 1) The two accessions of *S. hirtum* grouped together and formed the basal branch in the Lasiocarpa clade. 2) A large clade was identified consisting of all accessions of *S. vestissimum*, *S. hyporhodium*, *S. felinum*, *S. quitoense*, *S. lasiocarpum*, *S. repandum*, *S. candidum*, and *S. pseudolulo*. This was sister to the *S. pectinatum-S. stramonifolium-S. sessiliflorum* clade described above. Within this clade, *S. vestissimum* S432 formed the basal lineage, which was sister to the rest

TABLE 2. Length of *trnT* to *trnF* region in studied taxa. Values are raw sequence length in base pairs, not including indels in the final aligned version. [a] NA = Not available. First ca. 15 to 19 bp of sequence not readable.

Taxon	*trnT–trnL* spacer	*trnL* 5′ exon	*trnL* intron	*trnL* 3′ exon	*trnL–trnF* spacer	Total length (*trnT–L* to *trnL–F* spacers)
Solanum* section *Lasiocarpa						
S. candidum S249	734	35	497	50	524	1840
S. candidum 2898	734	35	497	50	524	1840
S. felinum 8915	734	35	497	50	677	1993
S. hirtum S404	738	36	497	50	518	1839
S. hirtum 730	717	36	497	50	459	1759
S. hyporhodium 717	734	35	497	50	677	1993
S. hyporhodium 8214	734	35	497	50	622	1938
S. lasiocarpum 9605	752	35	497	50	524	1858
S. pectinatum 8512	749	36	497	50	676	2008
S. pectinatum 2899	749	36	497	50	676	2008
S. pseudolulo 4276	734	35	497	50	677	1993
S. pseudolulo 995	734	35	497	50	524	1840
S. quitoense 2873	734	35	497	50	524	1840
S. quitoense 996	734	35	497	50	524	1840
S. repandum 8215	734	35	497	50	524	1840
S. repandum 8627	734	35	497	50	519	1835
S. sessiliflorum 458	749	36	497	50	523	1855
S. sessiliflorum 8255	749	36	497	50	523	1855
S. stramonifolium 860	716	36	497	50	753	2052
S. stramonifolium 154	716	36	497	50	700	1999
S. vestissimum 456	734	35	497	50	524	1840
S. vestissimum S432	734	35	497	50	523	1839
Solanum* subgenus *Leptostemonum						
S. acerifolium	761	36	497	50	639	1983
S. capsicoides	752	36	497	50	525	1860
S. jamaicense	NA[a]	36	497	50	631	NA[a]
S. mammosum	731	36	497	50	519	1833
S. melongena	NA[a]	36	497	50	462	NA[a]
S. palinacanthum	716	36	497	50	526	1825
S. robustum	710	36	497	50	567	1860
S. sisymbriifolium	717	35	497	50	623	1922
S. stagnale	710	36	497	50	462	1755
S. tenuispinum	717	36	497	50	541	1841
S. torvum	806	35	497	50	567	1955
S. wendlandii	723	36	497	50	404	1710
Outer outgroups						
S. abutiloides	712	36	497	50	409	1704
S. dulcamara	677	36	497	50	413	1673
S. luteoalbum	714	36	501	50	400	1701
S. pseudocapsicum	NA[a]	35	497	50	401	NA[a]

of the clade. 3) Within the clade described in #2 above, the two accessions of *S. quitoense* grouped together with 62–63% bootstrap support. 4) Also within this larger clade, *S. repandum* 8627 and both accessions of *S. pseudolulo* formed a lineage. This grouping received 60–63% bootstrap support.

The 50% majority rule consensus trees from analyses 3 and 4 (i.e., those that included indels as coded presence/absence characters) differed only in the pattern of relationships among members of section *Acanthophora* in the Leptostemonum clade; ingroup relationships were identical (Fig. 3). These analyses resolved the same clades as those described above with the following exceptions: 1) The two accessions of *S. hirtum* did not cluster as a monophyletic group, but instead formed a grade at the base of the Lasiocarpa clade. 2) The *S. stramonifolium* clade emerged as sister to a group consisting of *S. sessiliflorum* plus *S. pectinatum*. 3) The two accessions of *S. hyporhodium* plus *S. felinum* formed a monophyletic group within the large clade described in #2 above.

In general, adding the coded indel characters increased resolution on the majority rule consensus trees but decreased it slightly with respect to the strict consensus trees (i.e., in comparisons between Analyses 1 vs. 4 and 2 vs. 3). This is because the indels exhibit a fair amount of homoplasy, a result also seen by mapping the coded indel characters onto the trees from

TABLE 3. Characteristics of the *trn* sequence data matrix and parsimony results when each region is analyzed separately. Search parameters described in text except: [a] 500 random addition replicates, with no more than 150 trees ≥ 92 steps saved per replicate. [b] 500 random addition replicates, with no more than 200 trees ≥ 55 steps saved per replicate.

	trnT–trnL spacer	*trnL* 5′ exon	*trnL* intron	*trnL* 3′ exon	*trnL–trnF* spacer	Total length (*trnT–L* to *trn L–F* spacers)	Coded indels
Aligned length including gaps (bp)	910	36	505	50	833	2334	32
# variable characters	77	0	41	0	94	212	32
# parsimony-informative characters	25	0	11	0	39	75	32
# most parsimonious trees (length)	>73352 (92)[a]	—	67 (44)	—	28402 (123)	1907 (263)	>59042 (55)[b]
# nodes resolved in strict consensus tree (# ingroup nodes)	9 (4)	—	7 (2)	—	5 (1)	12 (5)	5 (1)
RI (CI excluding autapomorphies)	0.886 (0.778)	—	0.952 (0.857)	—	0.929 (0.778)	0.909 (0.761)	0.862 (0.593)
Aligned length excluding gaps (bp)	578	35	494	50	386	1543	
# variable characters	61	0	40	0	66	167	
# parsimony-informative characters	20	0	11	0	26	57	
# most parsimonious trees (length)	170 (75)	—	64 (43)	—	364 (89)	796 (210)	
# nodes resolved in strict consensus tree (# ingroup nodes)	5 (2)	—	6 (1)	—	7 (2)	12 (5)	
RI (CI excluding autapomorphies)	0.892 (0.767)	—	0.952 (0.857)	—	0.930 (0.750)	0.913 (0.747)	

sequence data alone (data not shown). About 25 to 50% of the coded indel characters were homoplastic when mapped onto the various trees. This is also reflected in the lower CI and RI values for the coded indel data set as compared to the other separately-analyzed regions of the *trn* data set (Table 3).

The various non-coding regions of the *trn* data set provided different numbers of phylogenetically informative characters and different levels of phylogenetic resolution (Table 3). When analyzed separately, the *trnL-trnF* spacer region including gaps provided the largest number of phylogenetically informative characters, yet this region resolved just one to two nodes within section *Lasiocarpa*. Of the individual regions of the *trn* array, the *trnT-trnL* spacer provided the greatest resolving power for both ingroup and outgroup nodes (Table 3). However, even greater resolution was achieved by including data from the two spacers plus the intron (Table 3), regardless of whether gaps were included or excluded from the data matrix.

DISCUSSION

Utility of* trn *Sequence Data. Of the non-coding regions sampled here, the *trnL-trnF* spacer was the most variable and provided the largest number of potentially phylogenetically informative characters. However, the *trnL-trnF* spacer alone did not provide resolving power over the entire phylogeny. Use of the *trnL-trnF* spacer sequence data alone resolved only five to seven nodes in the strict consensus trees, versus 10 to 12 nodes for the complete sequence data sets. Furthermore, *trnL-trnF* spacer data alone resolved just one to two nodes in the ingroup, supporting the monophyly of the twelve *Lasiocarpa* species and placing the two accessions of *S. sessiliflorum* as sister taxa. Thus, although the *trnL-trnF* spacer is a popular choice for phylogenetic reconstruction in many plant groups (e.g., Taberlet et al. 1991; Gielly and Taberlet 1994; van Ham et al. 1994; Kim et al. 1996), the greatest resolution in the *Lasiocarpa* study was provided by data from the entire *trn* array. It is difficult to predict from character variability or sequence divergence values alone what or how much sequence data will be desirable in examining a phylogenetic problem.

***Circumscription and Monophyly of Section* Lasiocarpa**. The twelve species traditionally considered to belong to *Solanum* section *Lasiocarpa* emerge as a monophyletic group in all analyses. The cpDNA data show that *S. stagnale* and *S. robustum* are not closely related to other members of section *Lasiocarpa*. *Solanum stagnale* was originally included in section *Lasiocarpa* in the monograph of Whalen et al. (1981), but at that time it was only known from several nineteenth century herbarium collections. Its large repand leaves and stellate-pubescent fruits were thought to unite *S. stagnale* with the rest of the species in section *Lasiocarpa*, although Whalen et al. (1981) regarded it as ''phylogenetically isolated'' and morphologically anomalous within the section. Whalen (1984) later removed *S. stagnale* from section *Lasiocarpa* and surmised that it was more closely related to taxa of his *S. polytrichum* group, although it is anomalous within that group due to its pubescent fruits and unarmed, weakly accrescent calyces. The *trn* data show that *S. stagnale* is not closely

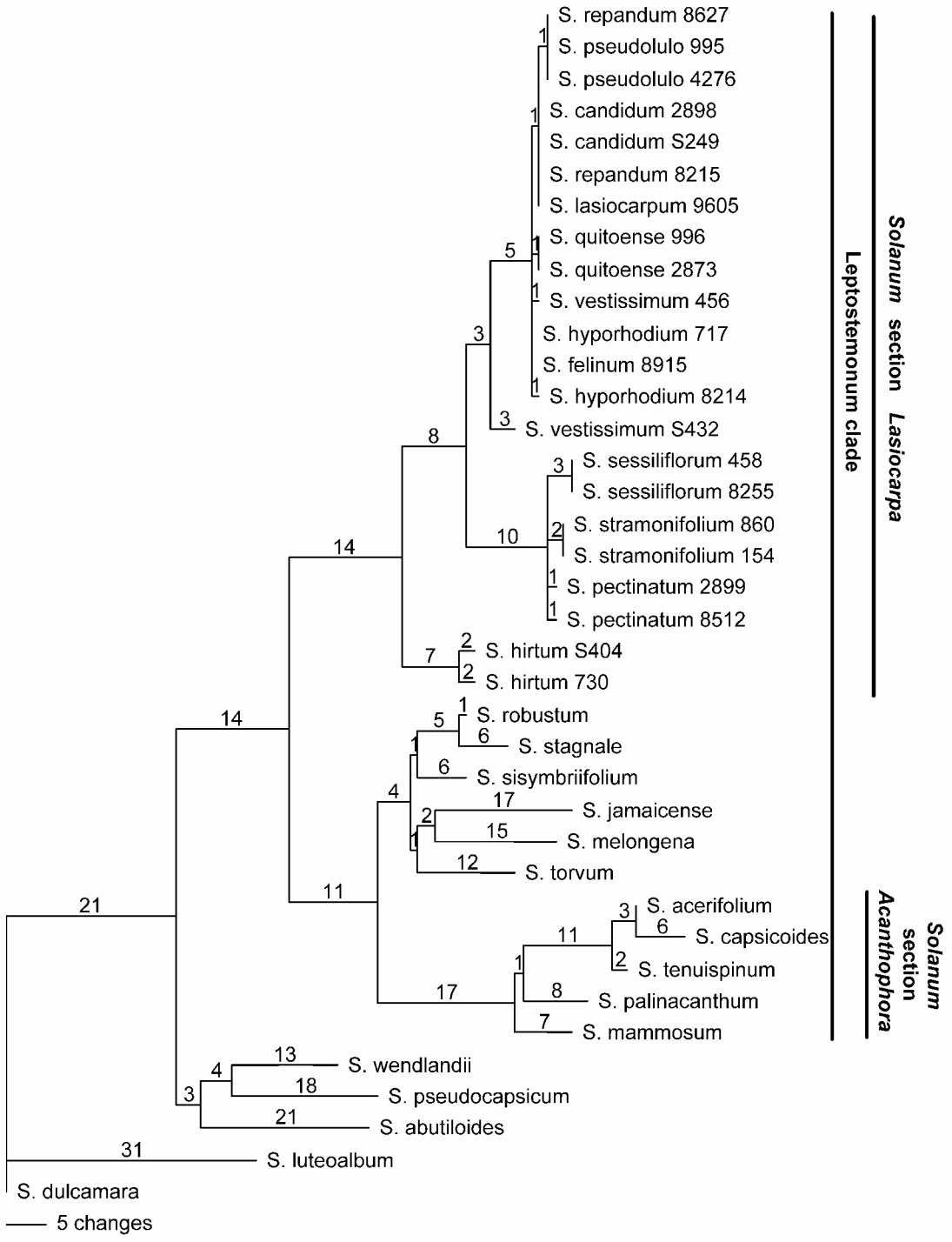

FIG. 1. One of 1907 most parsimonious trees of 263 steps from the complete aligned sequence data set (Analysis 1). Number of nucleotide changes is indicated above the branches.

related to members of section *Lasiocarpa*, but rather is sister to *S. robustum*, a species not included in the original *Lasiocarpa* monograph but placed within the section by Dunal (1852), Morton (1976), and Hunziker (2001). Whalen (1984) tentatively considered *S. robustum* to belong to the *S. erythrotrichum* species group, which also has pubescent berries. Although the *trn* data resolve *S. stagnale* and *S. robustum* as sister taxa

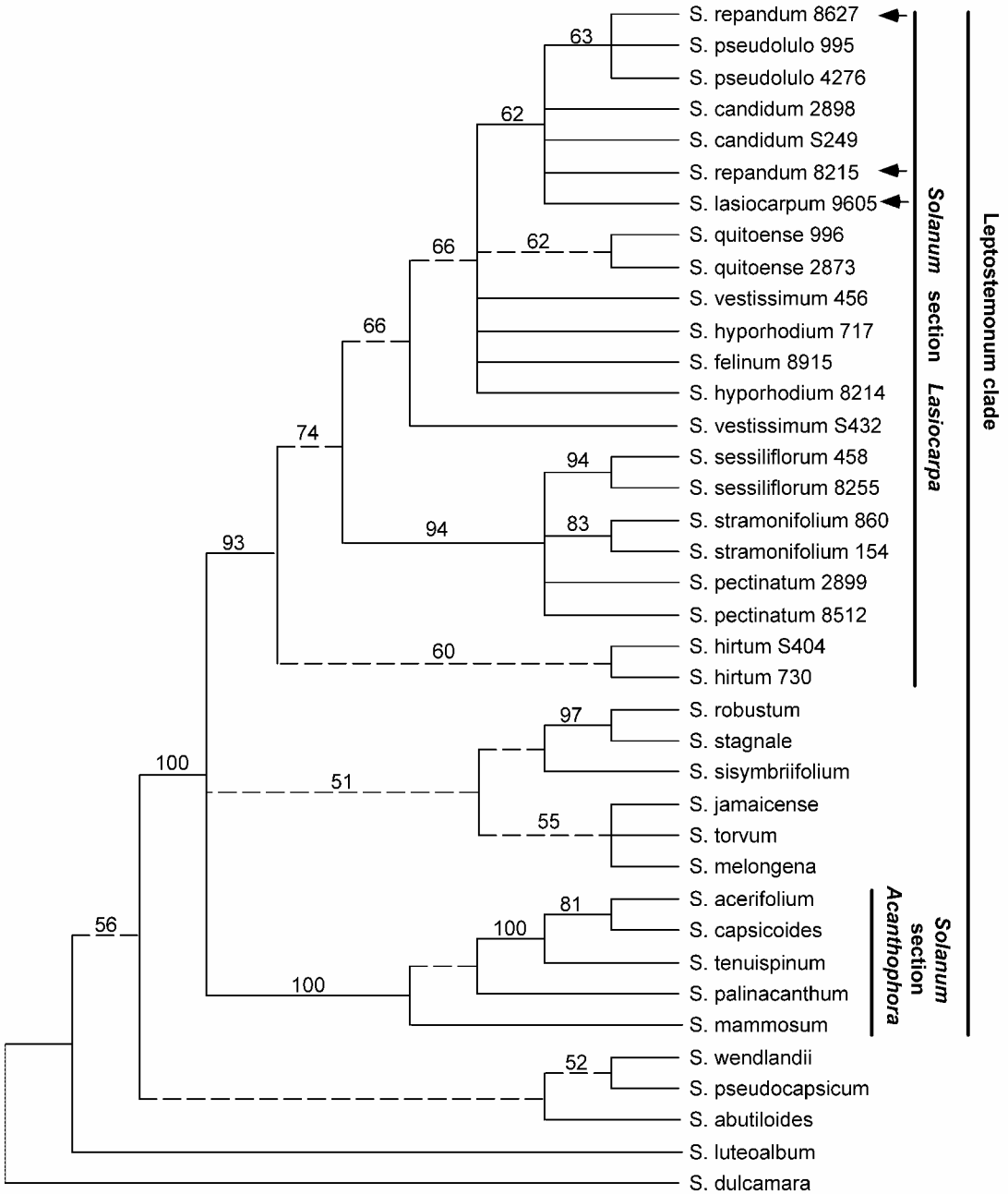

FIG. 2. 50% majority rule consensus tree from the complete aligned data set (Analysis 1). Dashed lines are branches that collapse in the strict consensus tree. Bootstrap values (500 replicates) included on the branches. Arrows mark Asian species of section *Lasiocarpa*; all other members of the section are New World taxa.

and thus suggest that they are more closely related than Whalen (1984) believed, more extensive sampling within *Solanum* subgenus *Leptostemonum* is needed to confirm this conclusion. Likewise, further sampling is necessary to identify the closest relatives to section *Lasiocarpa* within the Leptostemonum clade.

***Relationships Within* Solanum *Section* Lasiocarpa.** Three groups of species can be discerned within section *Lasiocarpa*. One consists solely of the two accessions of *S. hirtum*, which form either a basal grade or clade in the section. *Solanum hirtum* is the most widespread and variable species in section *Lasiocarpa* (Whalen et al. 1981) and can be distinguished from other members of the section by its relatively diminutive leaves and fruits and by its reflexed calyx lobes. Cladistic analyses of morphological and allozyme

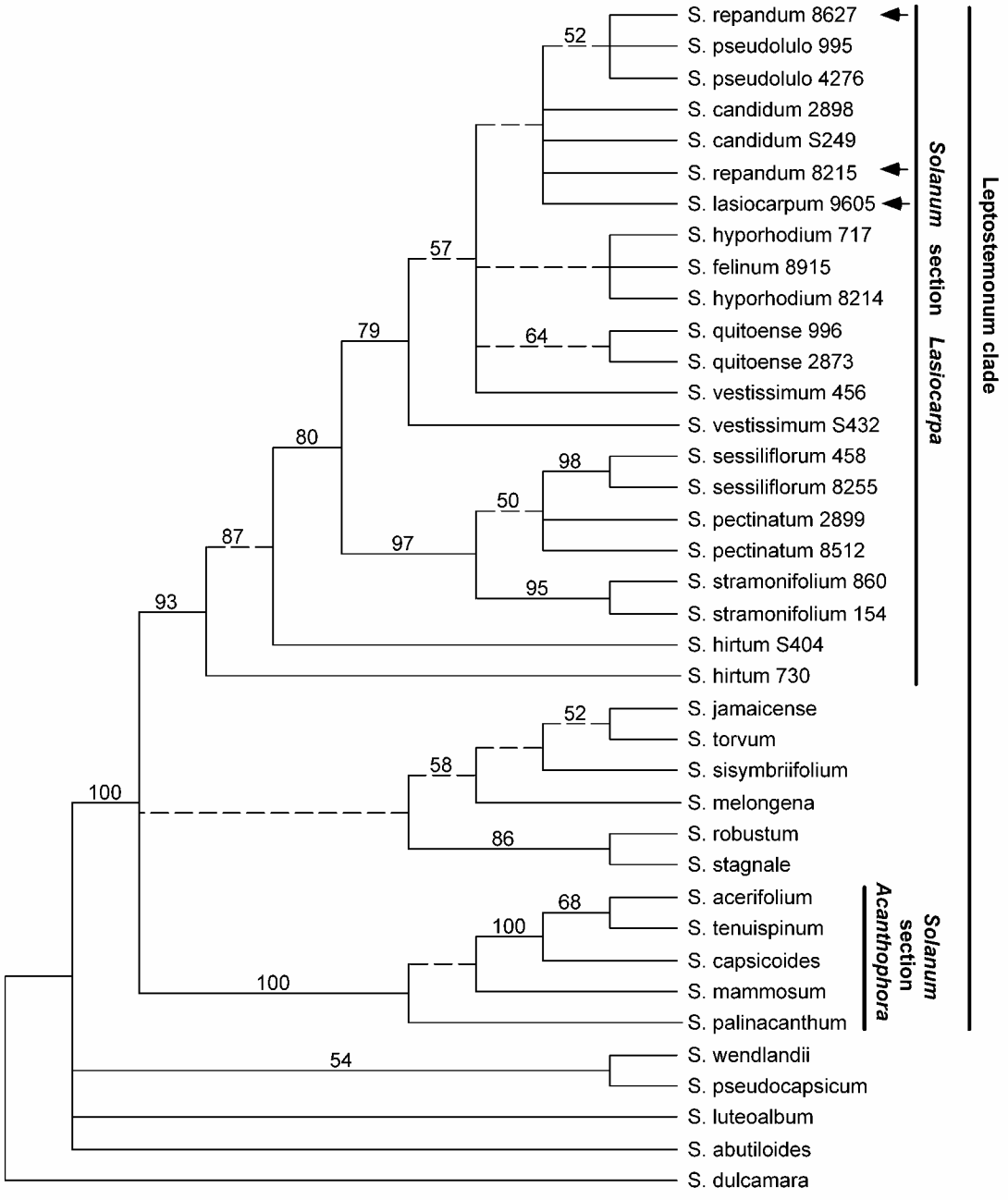

FIG. 3. 50% majority rule consensus tree from the aligned data set with indels excluded and coded indels included (Analysis 3). Dashed lines are branches that collapse in the strict consensus tree. Bootstrap values (500 replicates) included on the branches. Arrows mark Asian species of section *Lasiocarpa*; all other members of the section are New World taxa.

characters by Whalen et al. (1981) and Whalen and Caruso (1983) showed *S. hirtum* to belong to a clade including *S. lasiocarpum*, *S. candidum*, *S. quitoense*, and *S. pseudolulo*, and this relationship was recovered in a subset of the analyses of Bruneau et al. (1995) based on morphological and isozyme characters and chloroplast DNA restriction sites. *Solanum hirtum* hybridizes with *S. quitoense* (easily), with *S. stramonifolium* (with moderate success), and with *S. pseudolulo* (with difficulty) in greenhouse crossing trials (Heiser 1972, 1989), but no successful intraspecific crosses were obtained between accessions of *S. hirtum* from Trinidad and Costa Rica (Heiser 1972). Results from the *trn* data indicate that the sequences of the two accessions of *S. hirtum* (from Costa Rica and Ecuador) are very similar and that *S. hirtum* forms an isolated basal branch in

section *Lasiocarpa*. These findings are at odds with previous data from morphological, isozyme, and crossing studies, although there is some suggestion of this phylogenetic position from the analysis of cpDNA restriction site data in Bruneau et al. (1995). Bernardello et al. (1994) found that chromosomes of *S. hirtum* were most similar in morphology to *S. pectinatum*, but the *trn* data place *S. hirtum* and *S. pectinatum* on distinct clades.

The second clade resolved in the *trn* analyses consists of *S. pectinatum*, *S. stramonifolium*, and *S. sessiliflorum*. These taxa have been considered to be phylogenetically isolated from each other and from other members of the section based on morphological and karyotypic analyses as well as crossing studies (Heiser 1972, 1989; Whalen et al. 1981; Whalen and Caruso 1983; Bernardello et al. 1994). A clade containing *S. pectinatum*, *S. stramonifolium*, and *S. sessiliflorum* was also recovered in the analysis of cpDNA restriction site characters in Bruneau et al. (1995; their Fig. 1). Analyses of morphological and allozyme characters alone or in combination (Bruneau et al. 1995) as well as karyotype analyses (Bernardello et al. 1994) failed to support this relationship. However, when the morphological and allozyme characters were combined with the cpDNA restriction sites the three species formed a basal grade sister to the remaining species of section *Lasiocarpa* (Bruneau et al. 1995). *Solanum pectinatum*, *S. stramonifolium*, and *S. sessiliflorum*, along with *S. hirtum*, are low elevation species found generally below 1000 m. Thus, the *trn* data support the hypothesis of an early lowland radiation in section *Lasiocarpa* followed by diversification at middle and high elevations (Whalen et al. 1981; Whalen 1983; Whalen and Caruso 1983; Bruneau et al. 1995).

Solanum sessiliflorum, commonly known as the cocona, is cultivated for its large edible fruits. There is much variability in size, form, and flavor of the fruits and many locally named cultivars exist in South America (Schultes and Romero-Castañeda 1962), but these are all considered conspecific with *S. sessiliflorum* (Whalen et al. 1981). *Solanum sessiliflorum* var. *georgicum* (R. E. Schult.) Whalen differs from the typical variety in having spiny stems and leaves and small globose berries and is thought to perhaps represent the progenitor of the cocona. Although not nearly as important or widely used as *S. sessiliflorum*, *S. stramonifolium* also produces edible fruits and has both spiny and non-spiny forms, the latter formally recognized as *S. stramonifolium* var. *inerme* (Dunal) Whalen. Whalen et al. (1981) proposed that *S. sessiliflorum* and *S. stramonifolium* may be distantly related, but favored a closer relationship between *S. sessiliflorum* and *S. repandum* on the basis of phenetic and cladistic analyses of morphological data (Whalen et al. 1981; Whalen and Caruso 1983). However, Heiser (1987) and Bruneau et al. (1995) reanalyzed these data using more characters and better plant material and found that *S. repandum* was sister to *S. lasiocarpum*, not *S. sessiliflorum*. The *trn* data agree with the conclusions of Heiser (1987) and Bruneau et al. (1995) that *S. sessiliflorum* and *S. repandum* are not sister taxa and support the sister relationship between *S. sessiliflorum* and *S. stramonifolium*.

Solanum pectinatum is the third member of this well-supported clade. The relationships of this species have been enigmatic because it is the only member of the section with consistently unbranched hairs and it is reproductively isolated from other species of section *Lasiocarpa* (Heiser 1972, 1989). Because details of trichome morphology have been important in phylogenetic studies based on morphological characters, *S. pectinatum* was excluded from consideration in the morphological analyses of Whalen and Caruso (1983). However, the morphological analyses of Bruneau et al. (1995) included individuals of *S. pectinatum* reported to bear stellate trichomes. In these trees, *S. pectinatum* emerged on a clade along with *S. hirtum*, *S. candidum*, *S. quitoense*, *S. pseudolulo*, *S. felinum*, and *S. vestissimum*. Analyses of isozyme data gave a similar result (Whalen and Caruso 1983; Bruneau et al. 1995). The *trn* data conflict with this placement and are instead consistent with the cpDNA restriction site data in identifying a clade consisting of *S. pectinatum*, *S. sessiliflorum*, and *S. stramonifolium*.

The *S. pectinatum* 2899 accession from Santa Cruz, Bolivia used in the *trn* study is morphologically similar to typical *S. pectinatum* but bears short- to medium-stalked stellate hairs on the leaves and stem. The cauline hairs often bear gland-tipped midpoints that are longer than the lateral rays, and the rays themselves are divergently spreading to ascending. Exclusively unbranched trichomes are a hallmark of *S. pectinatum* and the 2899 accession came from a locality far to the southeast of other *S. pectinatum* collections (Whalen et al. 1981). However, *S. pectinatum* is occasionally cultivated for its edible fruits and thus could have been introduced to Bolivia by humans in recent times. Likewise, Bruneau et al. (1995) report that some specimens of *S. pectinatum* were found with sessile to short-stalked stellate stem hairs with spreading or ascending rays and midpoints as long as or longer than the lateral branches. The *trn* sequences from both *S. pectinatum* accessions were very similar. The taxonomic concept of *S. pectinatum* probably should be expanded to include variants with stellate trichomes.

The third clade resolved by the *trn* data includes *S. candidum*, *S. felinum*, *S. hyporhodium*, *S. lasiocarpum*, *S. pseudolulo*, *S. quitoense*, *S. repandum*, and *S. vestissimum*. *Solanum lasiocarpum* and *S. repandum* are native to the Old World; the remaining species are mainly montane taxa with a center of diversity in northwestern South America. This clade was also recovered by Bruneau et

al. (1995) in their analyses of cpDNA restriction sites and combined cpDNA, morphological, and isozyme data. However, their analyses of morphological and isozyme data, alone and in combination, placed *S. hirtum* within this clade, whereas this species forms an isolated basal branch in the *trn* trees. Data from crossing studies and karyotype analyses are equivocal with respect to support for this large group (Heiser 1972, 1989; Bernardello et al. 1994).

Within this large clade, coded indel data provide some support for the association of *S. hyporhodium* with *S. felinum*. In analyses without the coded indel data, all accessions of these two species along with *S. vestissimum* form a basal grade in the large clade described above, with *S. vestissimum* S432 comprising the basal branch in the entire large clade. All three of these taxa are high-elevation cloud forest species native to Venezuela and northern Colombia. Whalen et al. (1981) considered the three species to be closely related on morphological grounds. *Solanum felinum* and *S. vestissimum* are extremely similar morphologically, with *S. hyporhodium* less so (Bruneau et al. 1995). *Solanum hyporhodium* and *S. vestissimum* clustered together in phenetic and cladistic analyses of isozyme data (Whalen and Caruso 1983; Bruneau et al. 1995); *S. felinum* was not included in these studies. Crossing and karyotypic studies did not support a relationship among the three taxa (Heiser 1972, 1989; Bernardello et al. 1994), although *S. hyporhodium* and *S. felinum* had similar chromosome characteristics (Bernardello et al. 1994). Although the three taxa are closely associated in most of the *trn* trees, they do not form a monophyletic group. In addition, the S432 accession of *S. vestissimum* from Colombia is divergent from the other four representatives of the group, all of which are from Venezuela. Heiser (2001) noted that accessions identified as *S. vestissimum* from Colombia and Venezuela would not cross with each other and differed in their crossing behavior with *S. quitoense*. Further taxonomic work on species limits in this complex and more intensive sampling with more variable genes is warranted to ascertain the position of these high altitude Colombian and Venezuelan taxa.

Two questions that have been intensively studied with respect to this group of species concern the wild relatives of *S. quitoense* and the origin and relationships of the two Old World taxa of section *Lasiocarpa*. *Solanum quitoense*, the lulo or naranjilla, is a commonly cultivated fruit crop in Andean South America. Its range has recently spread to include Central America, where it is naturalized in Panama and Costa Rica. *Solanum quitoense* has been considered by some to be known only from cultivation, although spiny and feral forms exist in northwestern South America. Heiser (1972) proposed on morphological grounds that *S. quitoense* is most closely related to *S. candidum*, but the two species have different habitat preferences and hybridize only with difficulty. Although *S. quitoense* and *S. candidum* are not sister taxa in the *trn* trees, there is little character support and resolution in this area of the tree and a close relationship between the two taxa cannot be ruled out. However, the *trn* data refute hypotheses of close associations between *S. quitoense* and *S. hirtum*, *S. pectinatum*, *S. stramonifolium*, and *S. sessiliflorum*.

Likewise, the relationships of the two Asian disjuncts, *S. repandum* and *S. lasiocarpum*, have been a matter of debate. Whalen et al. (1981) and Whalen and Caruso (1983) suggested that *S. repandum* and *S. lasiocarpum* were not sister taxa, but instead that *S. repandum* was allied to and perhaps conspecific with *S. sessiliflorum*, whereas *S. lasiocarpum* was most closely related to *S. candidum*. Conversely, Heiser considered *S. repandum* and *S. lasiocarpum* to be closely related and perhaps conspecific (as *S. ferox*) and that *S. candidum* was sister to the Asian taxa (Heiser 1986, 1987, 1996). The *trn* data, as well as previous data from crossing and karytotype studies and analyses of cpDNA and morphological characters (Heiser 1986, 1987, 1996; Bernardello et al. 1994; Bruneau et al. 1995) supports the close relationship between *S. repandum* and *S. lasiocarpum* and thus Heiser's hypothesis. Furthermore, *S. candidum* emerges as a member of the *S. repandum*/*S. lasiocarpum* clade, conforming to Heiser's ideas of relationships. However, *S. repandum* and *S. lasiocarpum* did not form a monophyletic group in the *trn* analyses; rather, one accession of *S. repandum* formed a clade with the two *S. pseudolulo* accesssions. This result should not be over-interpreted, however, since there is little character support for the identification of lineages within the large clade that includes *S. repandum*, *S. lasiocarpum*, *S. pseudolulo*, *S. candidum*, *S. quitoense*, *S. hyporhodium*, *S. vestissimum*, and *S. felinum*.

In general, the *trn* trees are quite similar to those obtained from analyses of cpDNA restriction site data (cf. Fig. 1 in Bruneau et al. 1995). This is not surprising, given that the chloroplast genome is a single linked non-recombining genetic entity (Doyle 1992). Further molecular studies are underway using more variable nuclear genes in order to achieve better resolution of phylogenetic relationships among the species of section *Lasiocarpa*, to increase support for previously identified clades, and to compare phylogenies derived from maternally inherited chloroplast genes with those based on biparentally inherited nuclear markers.

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