A molecular phylogeny of *Solanum* sect. *Pteroidea* (Solanaceae) and the utility of COSII markers in resolving relationships among closely related species

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Abstract *Solanum* sect. *Pteroidea* is a lineage of ten species of neotropical herbs and vines with a center of distribution in the eastern Andean slopes. It is a member of the Potato clade of *Solanum*, a group that includes the potato (*S. tuberosum*) and tomato (*S. lycopersicum*). Members of *S.* sect. *Pteroidea* are characterized by inflorescences that emerge from the leaf axils and rugose, sharply pointed fruits in most species. The aim of this study is to infer phylogenetic relationships among sixteen species of *Solanum*, including all ten species of *S.* sect. *Pteroidea*, using DNA sequence data from the chloroplast *trnT-trnF* and five nuclear regions: ITS, the granule bound starch synthase gene (GBSSI or *waxy*), and three Conserved Orthologous Set II (COSII) markers. Results provide strong support for the monophyly of *S.* sect. *Pteroidea* and for its sister group relationship to *S.* sect. *Herpystichum*. *Solanum mite*, one of the most widespread and morphologically variable species in the section, is not monophyletic. Bayesian analyses using a total evidence concatenated approach and a coalescent approach implemented in BEST produced largely congruent topologies. The total evidence trees, however, were much more highly supported than the BEST trees. Although not useful individually in *S.* sect. *Pteroidea*, the three COSII markers were easy to amplify, provided clean sequences, and were tremendously useful in increasing resolution and support among the closely related species of *S.* sect. *Pteroidea* in combined analyses.

Keywords Bayesian estimation of species trees (BEST); Central America; COSII markers; phylogeny; *Solanum* section *Pteroidea*; South America

■ INTRODUCTION

The ten species of *Solanum* sect. *Pteroidea* Dunal form a relatively small but easily recognizable group among the approximately 1500 species of *Solanum* (Knapp & Helgason, 1997). Members of *S.* sect. *Pteroidea* are herbs and vines found in the understory of rainforests and cloud forests from southern Mexico to Bolivia, and are distinguished by inflorescences emerging from the leaf axils, conical and rugose fruits in many species, and unifoliate sympodial units (Fig. 1A–G). Inflorescences in *Solanum* are thought to be morphologically terminal, but in sect. *Pteroidea* fusion of petiole, stem, and inflorescence tissues result in inflorescences that appear to be axillary (Danert, 1967; Child, 1979). Most *Solanum* species have globose fruits with smooth surfaces. Globose fruits are found in some species of sect. *Pteroidea*, but most have sharply pointed fruits ranging from smooth to distinctly rugose. As a result of its morphological peculiarities, *S.* sect. *Pteroidea* has been considered to be somewhat isolated within *Solanum* and with enigmatic affinities (reviewed in Knapp & Helgason, 1997); however, recent molecular evidence suggests that it is a member of the informally named Potato clade (Bohs, 2005; Weese & Bohs, 2007).

Species of *S.* sect. *Pteroidea* have either simple or compound leaves. The simple-leaved species (*S. anceps*, *S. angustialatum*, *S. incurvum*) were not considered to be related to the compound-leaved species until Bitter (1912, 1921) grouped them together based on the axillary position of the inflorescences. Other morphological evidence, including inflorescence structure, the sharply pointed and rugose textured fruits, and floral characters, supports the grouping of simple- and compound-leaved species in a single clade (Knapp & Helgason, 1997).

In their revision of *S.* sect. *Pteroidea*, Knapp & Helgason (1997) divided the ten species of the section into two species groups (Table 1). The *S. ternatum* species group is characterized by large flowers (>1.2 cm in diameter) with petals planar at anthesis and flattened seeds that are reniform to rounded in shape (Fig. 1A–B). Members of the *S. mite* species group typically have smaller flowers (mostly <1 cm in diameter), petals strongly reflexed at anthesis, and plump, ovoid seeds (Fig. 1C–G). Also unique to the *S. mite* species group are the sharply pointed, coarsely rugose fruits not found among other species of *Solanum* (Fig. 1D, F).

The distribution of *S.* sect. *Pteroidea* is centered on the eastern slopes of the Andes in Ecuador and Peru, where all species but one are native (Table 1; see solanaceaesource.org for additional details). The remaining species, *S. trizygum*, occurs from Mexico into northern Venezuela and is the only species of sect. *Pteroidea* found in Central America. *Solanum anceps* and *S. mite* are the most widespread species in the section and extend from the Andes eastward into the Amazonian lowlands, with *S. anceps* reaching to the Guianas. Associated with its large range, *S. anceps* has tremendous variation in its stature, pubescence, pigmentation, and fruit shape and texture. On the other hand, *S. angustialatum* and *S. chamaepolybotryon* have extremely narrow ranges and are known from only a few morphologically homogeneous collections.

The goals of this study are to generate a molecular phylogenetic hypothesis for *Solanum* sect. *Pteroidea* in order to (1) assess the monophyly of the section and the phylogenetic relationships among its species; (2) examine the evolution of morphological features such as habit, fruit morphology, and leaf complexity in a phylogenetic context, and (3) evaluate the utility of Conserved Orthologous Set II (COSII) sequences, a relatively new category of markers, for phylogenetic inference. COSII markers are a set of single-copy, orthologous molecular markers found throughout the nuclear genome that can contain varying amounts of introns and exons and, consequently, have a wide range of variability and potential utility at many taxonomic levels. Wu & al. (2006) developed nearly 3000 primer pairs for COSII markers for use within the Asterid clade, and subsets of these have been used successfully for phylogenetic studies of several groups within the Solanaceae (Levin & al., 2009; Rodríguez & al., 2009). Here we combine three of these COSII markers with three of the markers used most successfully for phylogenetic inference in the Solanaceae: the nuclear ITS and GBSSI (or *waxy*), and chloroplast *trnT-trnF*.

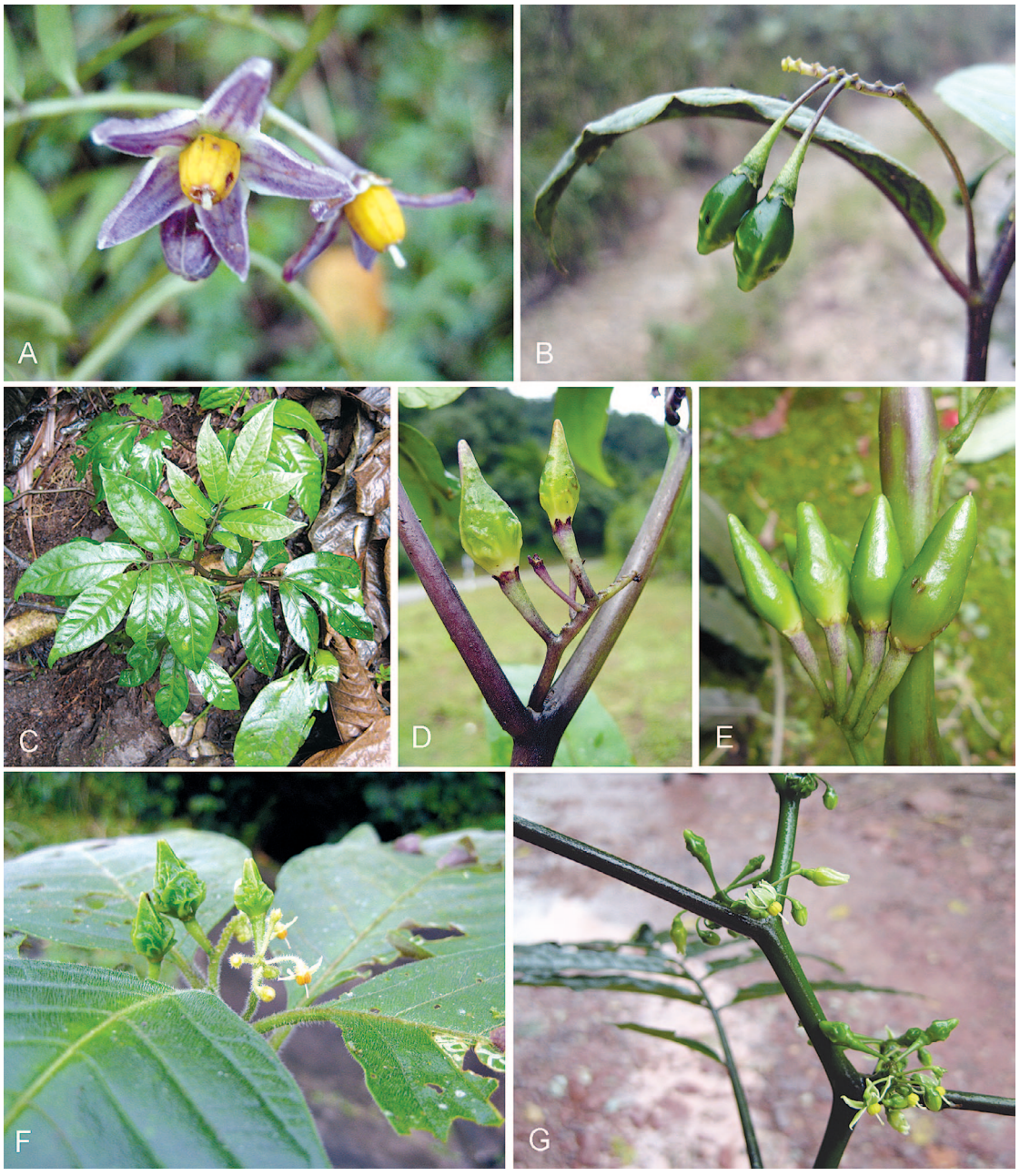

Fig. 1. Flowers, fruits, and habit of selected members of *Solanum* sect. *Pteroidea*. **A,** Flowers of *S. ternatum*; **B,** fruits of *S. incurvum* (immature); **C,** habit of *S. chamaepolybotryon*; **D,** fruits of *S. chamaepolybotryon*; **E,** fruits of *S. trizygum* (photo by S. Stern); **F,** flowers and fruits of *S. anceps*; **G,** flowers and fruits of *S. conicum*. Note the sharply conical fruits in *S. anceps* and *S. chamaepolybotryon*, and the rugose texture of the fruits in *S. incurvum*, *S. chamaepolybotryon*, and especially in *S. anceps*.

Table 1. Species of *Solanum* sect. *Pteroidea* and their geographic distributions (fide Knapp & Helgason, 1997).

Solanum sect. *Pteroidea*	Geographic distribution
***S. ternatum* species group**	
S. ternatum Ruiz & Pav.	Andes from Colombia and Venezuela to Bolivia; 100–2800 m
S. incurvum Ruiz & Pav.	Eastern slopes of the Andes from southern Ecuador to southern Peru; 1540–3000 m
***S. mite* species group**	
S. anceps Ruiz & Pav.	Colombia to Bolivia, Brazil, and the Guyana Shield; 100–3000 m
S. angustialatum Bitter	Northeastern Peru; 700–1200 m
S. chamaepolybotryon Bitter	North-central Peru; 950–3000 m
S. conicum Ruiz & Pav.	Southern Ecuador and southeastern Peru; 200–2000 m
S. mite Ruiz & Pav.	Eastern Colombia to Bolivia, throughout Brazil; 0–1500 m
S. savanillense Bitter	Southern Ecuador; 2300–3000 m
S. trizygum Bitter	Mexico to Venezuela; 600–3200 m
S. uleanum Bitter	Eastern slopes of the Andes from central Ecuador to central Peru; 200–1200 m

■ MATERIALS AND METHODS

Taxon sampling. — Twenty-eight accessions are included in this study for 16 species of *Solanum*, including all ten species of *Solanum* sect. *Pteroidea* recognized by Knapp & Helgason (1997; Table 1). When available, multiple accessions were sequenced from different parts of a species' range, including accessions of *S. anceps* and *S. mite* from Bolivia, Ecuador, and Peru and *S. ternatum* from Ecuador and Peru. In addition to sect. *Pteroidea*, we included outgroups representing all of the major clades within the Potato clade (Spooner & al., 1993; Weese & Bohs, 2007; E.J. Tepe & L. Bohs, unpub. manuscript) including representatives of *Solanum* sections *Anarrhichomenum* Bitter (*S. brevifolium* Dunal), *Basarthrum* (Bitter) Bitter (*S. caripense* Dunal), *Herpystichum* Bitter (*S. evolvulifolium* Greenm. and *S. phaseoloides* Pol.), *Lycopersicon* (Mill.) Wettst. (*S. lycopersicum* L.), and *Petota* Dumort. (*S. bulbocastanum* Dunal).

Molecular methods. — DNA was extracted from fresh or silica-dried leaves using the DNeasy plant mini extraction kit (Qiagen, Inc., Valencia, California, U.S.A.). Many species of *S.* sect. *Pteroidea* appear to have some secondary compounds that act as PCR inhibitors. For these difficult samples, we attempted to remove some of the problematic compounds by using either the additives to the Qiagen protocol suggested by Horne & al. (2004; sodium metabisulfate and dithiothreitol during DNA extraction) or a 2× CTAB buffer in place of Qiagen's AP1 buffer (Green & al., 1999).

PCR amplification followed the procedures described in White & al. (1990) and Vargas & al. (1998) for the internal transcribed spacer (ITS); Taberlet & al. (1991) for *trnT-trnF*; and Levin & al. (2005, 2006) for GBSSI. Because these traditional markers did not provide sufficient numbers of characters for well-resolved trees, we used sequences of three COSII markers (Wu & al., 2006; SOL Genomics Network, www.sgn.cornell.edu) shown to have excellent phylogenetic resolving power in the related *Solanum* sect. *Petota* (Rodríguez & al., 2009). The three markers used in this study were initially recommended to us by F. Rodríguez (pers. comm.) and were specifically selected because they amplified easily, produced single-banded PCR products in most accessions, and resulted in clean sequences without the need for cloning. Nomenclature of the COSII markers used in this study is taken from Rodríguez & al. (2009). PCR amplification of several accessions produced two bands for a single marker. In these cases, clean sequences were acquired by gel-isolation and direct sequencing of the band of the target size. Amplification of the three COSII markers was achieved using the following primers: cos1Cf-AGG TGC TTT CTT GTT TCT TCT TTC and cos1Cr-AGA GCA TAT CAC GAT ACT TGG TGT G, cos9Bf-TGG TGC AAC ACT TGT TGG TGT GG and cos9Br-TGG AGC CAG CCA TGC CAT TC, and cos11f-TTC TCT TTC CCT TAT CTG CAA CAC and cos11r-TCC TTC AAT CAT GTA CTT AGA GAC TTC. PCR reactions of 15 µL each contained 1.5 µL 10× Mg-free buffer, 1.5 mmol/L $MgCl_2$, 0.25 mmol/L dNTPs, 0.08 µmol/L of each primer, 0.7 µL DNA, and 1 unit of AmpliTaq Gold *Taq* polymerase (Applied Biosystems Inc., Foster City, California, U.S.A.). For recalcitrant samples, DNA stocks were diluted from 1/2 to 1/500 and/or additives were used in various combinations. The additives included 0.75 µL of a 50% glycerin/water solution, 0.75 µL DMSO, or 0.9 µL of a 10× PVP-40 solution. For the most difficult samples we used illustra PuRe Taq Ready-To-Go PCR Beads (GE Healthcare, Buckinghamshire, England). The thermal cycler program for the COSII markers was an initial 9 min at 94°C to activate the hot-start Amplitaq Gold, 35 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 2 min, ending with a 10 min extension at 72°C. PCR products were cleaned with the QiaQuick PCR Purification Kit (Qiagen, Valencia, California, U.S.A.) or the Promega Wizard SV Gel and PCR Clean-up system (Promega Corporation, Madison, Wisconsin, U.S.A.) and sequenced on ABI automated DNA sequencer at the University of Utah Core Facilities. Forward and reverse sequences were obtained for all samples, and contigs were assembled and proofread using Sequencher v.4.8 (GeneCodes, Ann Arbor, Michigan, U.S.A.). GenBank accession numbers of the DNA sequences used in this study are presented in the Appendix. Sequences of all markers were manually aligned using Se-Al v.2.0a11 (Rambout, 1996). Aligned datasets were submitted to TreeBASE (Study accession number = S2627; Matrix accession number = M5029–M5034).

Phylogenetic analyses. — Bayesian Inference (BI) analyses were performed using MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Parameters for the nucleotide substitution model were determined using MrModeltest v.2.2 (Nylander, 2004). The models were selected using the Akaike information criterion (AIC) because this method has been shown to perform better than the hierarchical likelihood ratio test when comparing nested models (Posada & Buckley, 2004). The selected models for all data partitions are

Table 2. Descriptive statistics for the datasets analyzed. Names for the COSII markers across the top of the table are from Rodriguez & al. (2009); designations for the COSII markers from the Sol Genomics Network (SGN; Mueller & al., 2005) are given in the first row.

	ITS	GBSSI	*trnT-trnF*	cos1C	cos9B	cos11	ITS + GBSSI + *trnT-trnF*	COS concatenated	All markers concatenated
SGN designation for COSII[a]	–	–	–	C2At1g13380	C2At3g03100	C2At5g14320	–	–	–
Raw sequence length	639–715	1733–1780	1609–1650	626–705	704–1666	414–489	–	–	–
Aligned length	747	2238	1714	731	2396	693	3820	4699	8519
PI sites (percent)	109 (14.6)	103 (4.6)	35 (2.0)	64 (8.8)	95 (4.0)	75 (10.8)	234 (6.13)	247 (5.26)	481 (5.65)
Range of pairwise distances	0–0.0946	0–0.0194	0–0.0113	0–0.0317	0–0.0413	0–0.0785	0–0.04166	0–0.023	0–0.3108
% intronic content of COSII	–	–	–	66%–70%	78%–83%[b]	67%–72%	–	–	–
Maximum parsimony analyses									
No. of MPT	2286	145	3	12	1	41,413	96	300	146
Length of MPT	281	145	49	95	141	117	452	364	933
CI, RI	0.59, 0.64	0.83, 0.92	0.78, 0.92	0.77, 0.85	0.79, 0.87	0.77, 0.88	0.63, 0.75	0.71, 0.81	0.64, 0.75
Bayesian analyses									
Model	GTR+G	GTR+G	GTR+I	GTR+G	HKY+G	GTR+I	GTR+I+G	GTR+G	GTR+I+G
No. of generations	1,887,000	532,000	678,000	525,000	527,000	991,000	996,000	1,146,000	72,000
No. of generations removed as burn-in	50,000	10,000	5,000	50,000	15,000	5,000	10,000	10,000	10,000
Within *Solanum* sect. *Pteroidea*									
No. resolved nodes in BI consensus/ MP strict consensus trees	10/13	12/12	11/11	14/9	11/14	9/11	17/7	14/9	19/18
No. of supported nodes (≥0.95 PP and ≥90% BP)	1	2	3	5	3	3	4	7	9

CI, consistency index; MPT, most parsimonious trees; PI, parsimony informative; RI, retention index. MPT, CI, and RI are reported with uninformative sites excluded.

[a] The names for the COSII markers used throughout this paper were taken from Rodriguez & al. (2009).

[b] Percentages calculated excluding two large autapomorphic insertions; intronic content is 78%–91% when insertions are included.

presented in Table 2. Using random starting trees, MrBayes was run until the average standard deviation of the split frequencies of two simultaneous runs reached 0.01, with one tree sampled every 1000 generations. The analyses were performed using the parallel version of MrBayes v.3.1.2 on the freely available Bioportal (www.bioportal.uio.no) with 4–10 chains per run and all other settings as the defaults. Post analysis was carried out in the serial version of MrBayes v.3.1.2 to determine the number of trees to omit as burnin and to compute the consensus tree and posterior probabilities (PP).

Maximum parsimony (MP) analyses were performed on each dataset separately and on the concatenated matrices using PAUP* v.4.0b10 (Swofford, 2003). All characters were weighted equally and gaps treated as missing data in full heuristic analyses with 100 random addition sequence replicates, TBR swapping, Steepest Descent, and all other settings kept as the defaults. Bootstrap (BP) values for nodes were estimated from full heuristic searches of 5000 replicates with MaxTrees set at 10,000 and TBR branch swapping.

Prior to combining the individual datasets, a partition homogeneity test (PHT) test (Farris & al., 1994) was run to test for incongruence using PAUP*. The PHT was implemented using 100 replicates with 10 random addition sequences per replicate and rearrangements limited to 1,000,000 per replicate. In addition, because this test has been shown to suffer from type I errors when phylogenetic signal is low (Dolphin & al., 2000; Yoder & al., 2001; Darlu & Lecointre, 2002; Dowton & Austin, 2002; Hipp & al., 2004), phylogenies of the nuclear and chloroplast datasets were compared to each other to detect areas of well-supported incongruence (i.e., differences supported by high bootstrap values and/or posterior probabilities; Seelanan & al., 1997; Wiens, 1998). Following Wiens (1998) criteria for combining matrices, we compared BI trees from the individual markers to each other and to the consensus tree of the concatenated analysis and identified all cases in which incongruence between markers was supported by PP ≥ 0.95 and BP ≥ 90. This procedure was repeated for the MP trees. Some nodes had high PP, but low BP support (PP values are often inflated relative to BP; Cummings & al., 2003; Erixon & al., 2003; Simmons & al.,

2004), so throughout this study we conservatively considered supported nodes to have both high PP (i.e., statistical support of 0.95 and above) and high BP (90 and above).

Since some accessions were missing all or part of one or more markers, we also analyzed a reduced 13-accession dataset in order to minimize the potential effects of missing data. The reduced dataset included one accession per species, except for *S. mite* for which we included two accessions since this species was not monophyletic in analyses of the complete data matrix. The thirteen accessions chosen for the reduced dataset were those with the most complete data for all regions. For *S. mite* we included one accession from each of the separate clades of this species. Because *Solanum* sect. *Herpystichum* was sister to sect. *Pteroidea* in all analyses of the full 28-accessions dataset, *S. evolvulifolium* and *S. phaseoloides* were used as the outgroup in the 13-accession dataset.

We analyzed the 13-accession combined datasets in two different ways: using a concatenated dataset, and a coalescent approach using Bayesian Estimation of Species Trees (BEST v.2.3; Liu, 2008). Rather than considering the separate markers together as in a concatenated analysis, BEST simultaneously generates gene trees from the individual markers and a species phylogeny based on the individual gene topologies. BEST analyses were conducted using the same models found for each target region as above, and were run for 10,000,000 generations with four chains, with all other parameters kept as the program defaults. The burn-in values were determined and consensus trees constructed in BEST using the sump and sumt commands. Because missing data can result in spurious results from Bayesian analyses (E.J. Tepe, pers. obs.) we did not run BEST on the 28-accession combined dataset to avoid potentially misleading results.

Because our analyses did not recover the same species groups found by Knapp & Helgason (1997), we compared unconstrained trees of the six-marker 28-accession dataset to those in which members of each of the two species groups were constrained to be monophyletic with the Shimodaira-Hasegawa test (SH; Shimodaira & Hasegawa, 1999) option in PAUP* using RELL bootstrap with 1000 pseudoreplicates and the same model parameters as above. The log-likelihood scores of the constrained trees were compared to those of the unconstrained MP trees, 10 randomly chosen post-burn-in BI trees, and the BI consensus tree.

■ RESULTS

Complete sequences were obtained for most of the 22 ingroup and six outgroup accessions for most of the six markers (total missing data ca. 3.4%; Appendix). All concatenated and BEST analyses strongly support the monophyly of *Solanum* sect. *Pteroidea*, and of all species with multiple accessions except for *S. mite* (Figs. 2 and 3A–F). The two accessions of *S. mite* from Bolivia (2750, 3014) are always resolved apart from the accessions from Ecuador and Peru (2364, 3627). *Solanum* sect. *Herpystichum*, represented here by *S. evolvulifolium* and *S. phaseoloides*, was strongly supported as sister to *S.* sect. *Pteroidea* in analyses of the 28-accession concatenated dataset (1.0 PP, 100 BP; Fig. 2), and in the concatenated 28-gene datasets of ITS+GBSSI+*trnT-trnF* and the COSII markers combined (0.81–1.0 PP, 70–100 BP; Fig. S1 in the Electronic Supplement to the online version of this article). This relationship was also supported by individual analyses of ITS, GBSSI and *trnT-trnF* (0.79–1.0 PP, 63–100 BP), but not in those derived from the individual COSII markers (Fig. S2 in the Electronic Supplement). Details of the analyses are presented in Table 2.

Congruence of datasets. — Results of the PHT tests comparing all markers and all 28 accessions at once indicated significant incongruence ($P < 0.01$). Likewise, pairwise comparisons of ITS, GBSSI, and *trnT-trnF*, and of the COSII markers were also incongruent according to the PHT test ($P < 0.01$). Most markers taken alone resulted in poorly resolved and poorly supported trees and no two of the six markers produced the same topology. In no case did two trees from the separate analyses of each marker share the same well-supported incongruence (taken here as a node with PP $\geq$ 0.95 and BP $\geq$ 90) relative to the topology shown in Fig. 2. Comparison of the trees derived from individual analyses of ITS, GBSSI, and cos9B showed no instances of well-supported incongruence to the topology in Fig. 2. The *trnT-trnF* tree had support for a sister relationship between *S. incurvum* and *S. anceps*+*S. angustialatum*+*S. chamaepolybotryon*+*S. conicum*+the Andean accessions of *S. mite* (accessions 2364 and 3627; 1.0 PP, 96 BP; Fig. S2). The cos1C tree had a clade composed of one accession of *S. anceps* (2790a), *S. conicum*, *S. angustialatum*, and *S. chamaepolybotryon* (1.0 PP, 97 BP), and two nodes within a clade containing another accession of *S. anceps* (3626) and the two accessions of *S. ternatum* (1.0 PP, 94 and 99 BP; Fig. S2). Finally, cos11 had strong support for a large clade that contained all species of *S.* sect. *Pteroidea* except for *S. angustialatum*, *S. chamaepolybotryon*, and *S. trizygum* (Fig. S2). Because comparison of the individual trees revealed very few instances of hard incongruence (relative to the total number of nodes), all 28 accessions were included in a concatenated analysis of ITS, GBSSI, *trnT-trnF*, cos1C, cos9B, and cos11 (Fig. 2). In order to compare the relative amounts of resolution and support provided by combinations of the various markers, we also analyzed the three COSII markers together and the three regions ITS, GBSSI, and *trnT-trnF* together.

All six markers. — *Solanum* sect. *Pteroidea* was strongly supported as monophyletic in all concatenated and BEST analyses (1.0 PP, 100 BP; Figs. 2 and 3A–B). Its monophyly was moderately to strongly supported in most analyses of the individual markers (0.79–1.0 PP, 63–100 BP), except for cos1C in which *S.* sect. *Pteroidea* emerged in a polytomy with *S. evolvulifolium*, and cos11 in which several species of sect. *Pteroidea* were nested within the outgroup (Fig. S2). The nodes separating the species of *S.* sect. *Pteroidea* in the cos11 tree, however, were poorly supported. Species with multiple accessions were monophyletic in the six-marker 28-accession analyses (i.e., all markers concatenated), except for *S. mite* (Figs. 2 and 3A–B). The Bolivian accessions of *S. mite* (2570, 3014) were moderately to strongly supported as sister to *S. incurvum* rather than to the two Andean accessions of *S. mite* from Ecuador and Peru (2364, 3627; Fig. 2).

Trees forcing all accessions of *S. mite* into a single clade were significantly different from the unconstrained BI and MP and trees ($P < 0.01$) according to the SH test. Details of markers analyzed individually are given below.

The BI and MP consensus trees from the six-marker 28-accession analyses were highly congruent and differed only in resolution (Fig. 2, arrows), and in the position of *S. uleanum*, which MP placed in a grade between *S. ternatum* and the rest of the species of *S.* sect. *Pteroidea* (BP < 50), and *S. savanillense*, which MP placed as sister to the rest of sect. *Pteroidea* (BP = 51). The following discussion is based on the results of BI analyses, because the MP results varied only slightly.

BEST analysis of the six-marker 13-accession dataset resulted in a well-resolved topology that was largely congruent with analyses of the concatenated dataset using BI. The single difference in topology between the concatenated and BEST analyses was the placement of *S. conicum* (Fig. 3A vs. 3B). Support, however, was considerably lower throughout the BEST tree. Themes common to all of the six-marker 13-accession analyses included close relationships between *S. incurvum* and the Bolivian accession of *S. mite*, and *S. ternatum* as sister to a clade containing *S. anceps*, *S. angustialatum*, *S. chamaepolybotryon*, *S. conicum*, *S. trizygum*, *S. uleanum* and the Andean accession of *S. mite*. Other common themes included a close relationship between *S. anceps* and *S. uleanum*, and between *S. angustialatum*, *S. chamaepolybotryon*, and *S. trizygum*. Trees constrained to conform to the *S. ternatum* and the *S. mite* species groups identified by Knapp & Helgason (1997) were not significantly different from unconstrained trees ($P = 0.09–0.1$).

ITS, GBSSI, and *trnT-trnF* sequence data. — The numbers of parsimony-informative (PI) characters were high relative to the COSII markers for ITS (109) and GBSSI (103), and low in *trnT-trnF* (35; Table 2). However, given the lengths of the sequences the percentages of PI characters were lower than the COSII markers for GBSSI (4.6%) and *trnT-trnF* (2%), but higher for ITS (14.6%). Phylogenetic analyses of these markers individually resulted in incongruent, poorly resolved and poorly supported topologies (Fig. S2). *Solanum* sect. *Pteroidea*

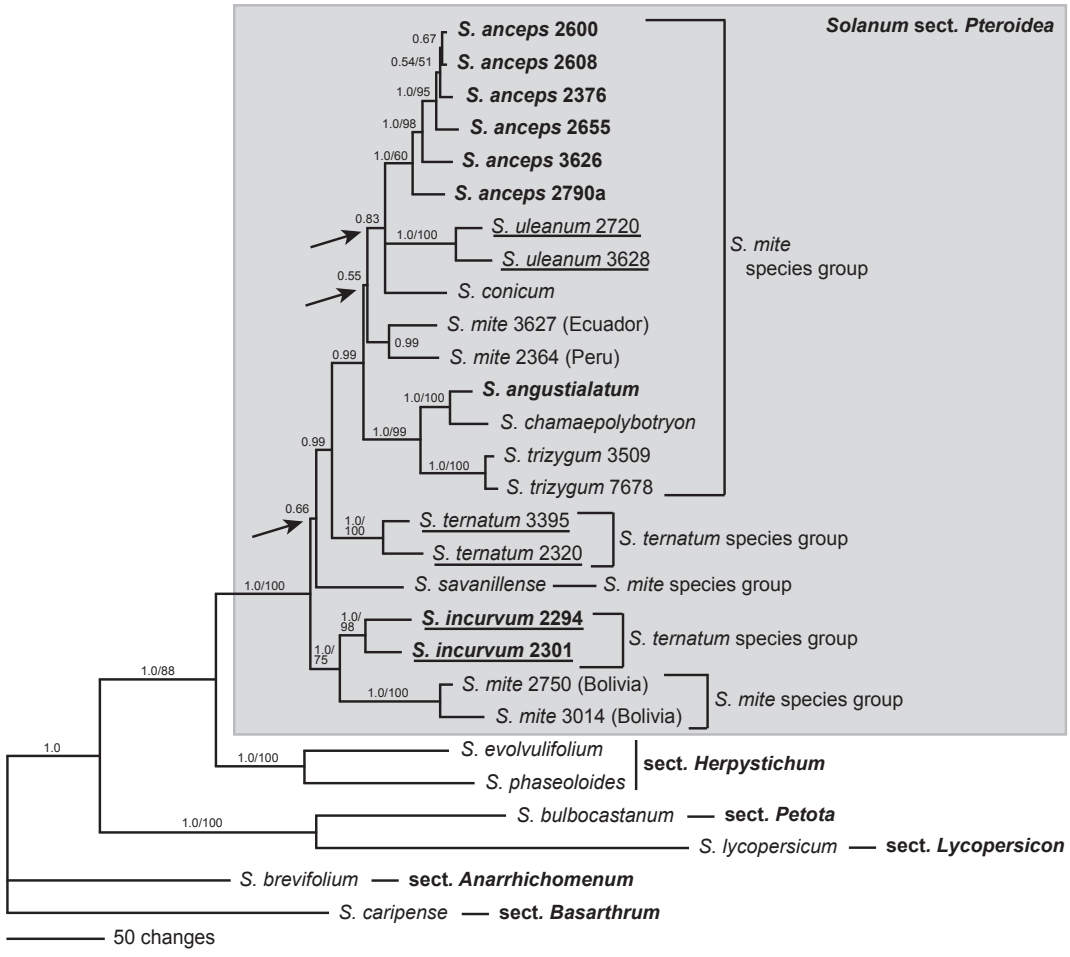

Fig. 2. 50% majority rule tree from Bayesian analysis of the concatenated six-marker 28-accession dataset including ITS, GBSSI, *trnT-trnF*, and the COSII markers cos1C, cos9B, and cos11. Branch support values are Bayesian posterior probability ≥ 0.5/maximum parsimony bootstrap ≥50%. Branches present in the Bayesian, but not the maximum parsimony analyses are indicated by arrows. Species of *Solanum* sect. *Pteroidea* are indicated by the gray box. The informal species groups follow Knapp & Helgason (1997). Multiple accessions of a species are designated by collection numbers given in the Appendix, and the geographic source of the four accessions of *S. mite* are given since this species is not monophyletic. Simple-leaved species are in bold and species with a viny habit are underlined.

was monophyletic in the individual analyses, as were *S. ternatum*, *S. trizygum* and the Bolivian accessions of *S. mite*. *Solanum uleanum* was nested among accessions of *S. anceps* in analyses of GBSSI; accessions of *S. anceps* were in a polytomy with other species in ITS and *trnT-trnF*. *Solanum uleanum* was monophyletic in the ITS and *trnT-trnF* datasets, but not in GBSSI. All other species with multiple accessions were parts of polytomies with other species. Both the GBSSI and *trnT-trnF* trees supported a close relationship between *S. angustialatum* and *S. chamaepolybotryon*.

The results of concatenated and BEST analyses for the combined 13-accession ITS+GBSSI+*trnT-trnF* data (Fig. 3C–D) were incongruent with each other and with the analyses of all markers combined (Fig. 3A–B). *Solanum conicum* was supported as sister to *S. anceps*+*S. uleanum* in the concatenated, but unsupported in the BEST analysis. The placement of *S. savanillense*, *S. ternatum*, and *S. trizygum* also differed between the two analyses. They were placed in a grade sister to the rest of *S.* sect. *Pteroidea* using BEST; in contrast, the clade formed by *S. incurvum*+Bolivian *S. mite* was sister to the rest of the section in the concatenated analysis. However, the positions of these species were poorly supported in all analyses of the ITS+GBSSI+*trnT-trnF* dataset.

COSII sequence data. — The numbers of PI characters in the COSII markers varied from 64 to 95 corresponding to 4% to 10.8% of aligned sequence length (Table 2). Cos9B had a 786 bp insertion in *S. conicum* and a 653 bp insertion in one accession of *S. uleanum* (2720), lowering the percentage of PI characters for that marker considerably. Without these two indels, the PI characters increased from 4% to 9.9% for cos9B. Intronic content for the COSII markers ranged from 66% to 91% (66%–83% when the two large insertions in cos9B are omitted).

Solanum sect. *Pteroidea* was monophyletic in MP and BI individual analyses of cos9B (PP 1.0), but not in cos1C or cos11 (Fig. S2 in the Electronic Supplement). *Solanum anceps* is monophyletic in all COSII individual analyses except for cos1C in which accessions were resolved in three separate clades. All other species for which we have multiple accessions are

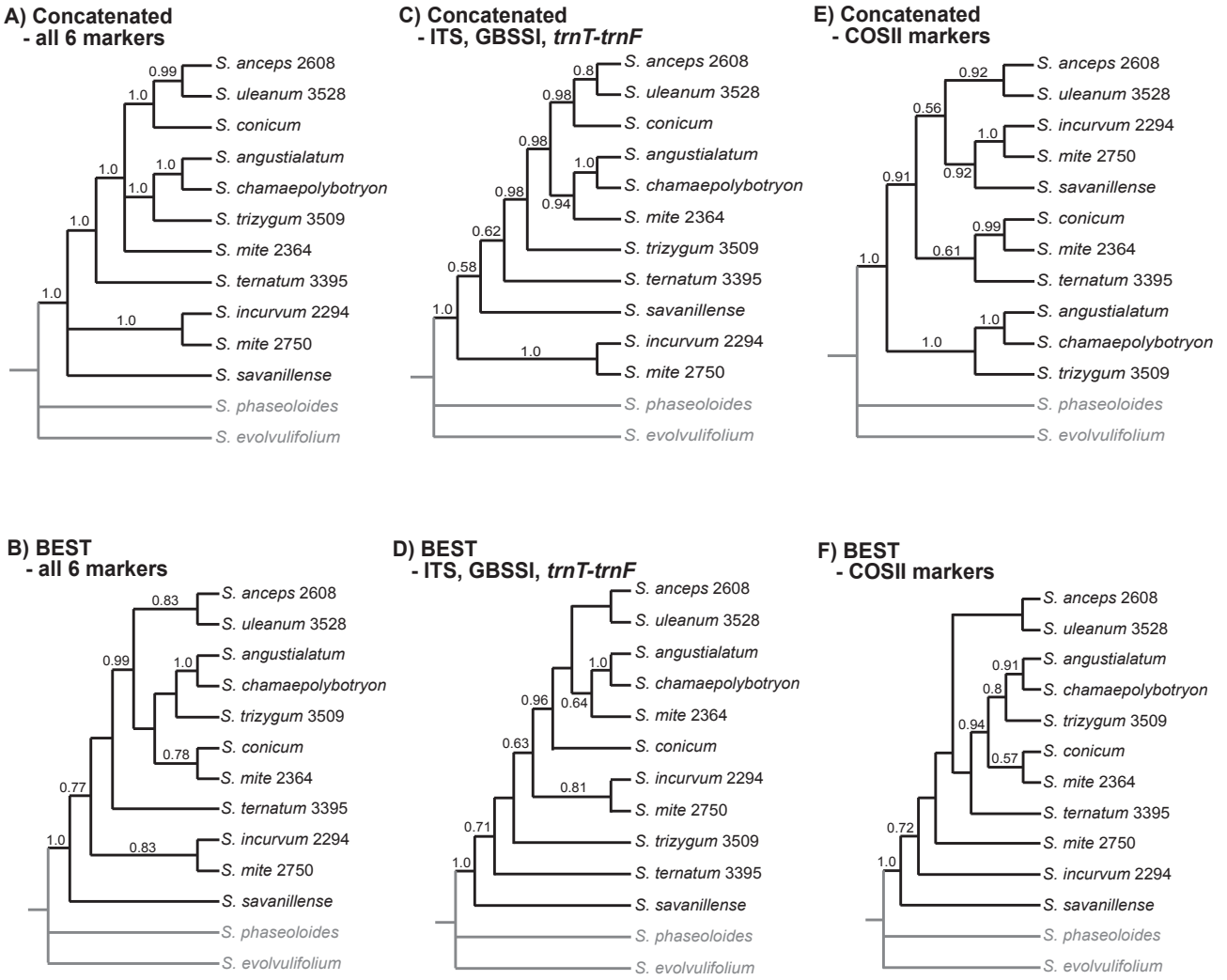

Fig. 3. 50% majority rule consensus trees from Bayesian analysis of the 13-accession concatenated datasets and from a coalescent approach implemented through BEST. Bayesian posterior probabilities ≥0.5 are shown above the nodes. *Solanum* sect. *Pteroidea* is in black; outgroup species (*Solanum* sect. *Herpystichum*) are in gray.

monophyletic or unresolved. Other themes apparent in the individual analyses are a close relationship between *S. incurvum* + Bolivian *S. mite*, *S. angustialatum* + *S. chamaepolybotryon*, and a tendency for these two species to form a clade with *S. trizygum*.

Concatenated and BEST analyses of the 13-accession COSII marker dataset differed strikingly from each other (Fig. 3E–F). The BEST analysis most reflected the major trends in the other analyses, but with very poor support for most nodes (Fig. 3F). The concatenated COSII topology is the most divergent of all analyses; however, most nodes are not well supported except for those observed in the other analyses, such as *S. anceps* + *S. uleanum*, *S. angustialatum* + *S. chamaepolybotryon* + *S. trizygum*, and *S. incurvum* + Bolivian *S. mite* (Fig. 3E).

■ DISCUSSION

Comparative utility of the six molecular markers. — The COSII markers were highly variable and provided numerous parsimony informative characters to our dataset. COSII markers have also proven useful in the closely related *Solanum* sections *Lycopersicon* and *Petota* (Rodríguez & al., 2009) and in *Lycium* (also Solanaceae; Levin & al., 2009). The COSII markers used in this study were chosen because of their length, their high intronic content, and because they produced single-banded PCR products. The percentages of PI characters in the COSII markers used here were inversely correlated with raw sequence length and were not directly related to the percent intronic content (Table 2). All three COSII markers had much higher percentages of PI sites than either GBSSI or *trnT-trnF* (when cos9B is considered without the two long, autapomorphic insertions), but lower than ITS (Table 2). Homoplasy (as measured by the consistency index), however, was much higher in ITS than in the COSII markers. Overall, GBSSI has the highest number of variable characters, but this region is long and must be sequenced in two segments to achieve full coverage with reasonable overlap. In contrast, the COSII markers were easy to amplify, sequenced cleanly, could be sequenced in a single pass with complete overlap in most cases, had relatively low homoplasy, and helped produce a well-resolved and highly supported phylogeny (Fig. 2). The COSII markers individually and combined with each other produced trees that were either poorly resolved, poorly supported, or that were largely in conflict with the trees produced by ITS + GBSSI + *trnT-trnF* (Fig. S1 in the Electronic Supplement), or by all six markers combined (Fig. 2). Thus, the COSII markers allowed us to efficiently increase the number of markers and PI characters in our dataset, but were most useful only when analyzed in combination with ITS, GBSSI, and *trnT-trnF*.

Incongruence among individual markers can result from methodology or biology. Methodological factors that can result in misleading topologies include sampling error, the wrong model of sequence evolution, long branches, a mix of long and short branches, undetected paralogy, or incorrect alignment (Huson & Bryant, 2006; Baum, 2007; Rodríguez & al., 2009). Other factors known to affect phylogenetic analyses that are not the result of differing histories of the markers include homoplasy, variation of sequence length and PI sites, and non-independence of nucleotide substitutions (Sullivan, 1996; Averof & al., 2000; Sanderson & Shaffer, 2002). If the discordance among the individual topologies is, in fact, due only to methodological issues, then the highly resolved and highly supported tree from the concatenated six-gene 28-accession analyses (Fig. 2) may be the result of hidden phylogenetic signal present in each of the individual matrices and revealed by concatenation, but overwhelmed by homoplasy or idiosyncratic artifacts of analysis when each gene is analyzed separately (Sullivan, 1996; Rokas & al., 2003; Gatesy & Baker, 2005).

The observed incongruence, however, may also be biological and result from varying genetic histories among the different markers. Under this scenario, each of the discordant gene trees may be accurate and reflect some biological process such as different evolutionary histories resulting from hybridization or introgression (Anderson & al., 2006; Prohens & al., 2006), allopolyploidy (Spooner & al., 2008; Rodríguez & Spooner, 2009), incomplete lineage sorting (Comes & Abbott, 2001; Liu & Pearl, 2007; Degnan & Rosenberg, 2009), or by evolution acting differently on different regions of the genome (Mason-Gamer & Kellogg, 1996). In this case, the concatenated topology may be considerably different from the species tree, and the BEST topology may be a more accurate reconstruction because this methodology allows for differing evolutionary histories of genes. Whether any of these biological processes are at work within *Solanum* sect. *Pteroidea* is currently unknown; however, the ten species are morphologically well-defined and easily distinguished and are thought to have none of the taxonomic complexities of *Solanum* sections *Basarthrum* or *Petota*, groups known to be complicated by these processes (Anderson & al., 2006; Prohens & al., 2006; Spooner, 2009). Furthermore, the possibility of polyploidy for the accessions included here is unlikely because direct sequencing returned clean, unambiguous sequences in all cases.

Higher level relationships. — *Solanum* sect. *Herpystichum*, represented here by *S. evolvulifolium* and *S. phaseoloides*, is strongly supported as sister to *S.* sect. *Pteroidea* in analyses of the six-marker 28-accession dataset (Fig. 2). Morphological similarities between the two sections include a tendency toward vininess, the presence of adventitious roots, and conical fruits (flattened in some species of sect. *Herpystichum*), with the key difference being the axillary placement of the inflorescences in sect. *Pteroidea* vs. terminal to extra-axillary in sect. *Herpystichum*. *Solanum* sect. *Herpystichum* is supported as monophyletic in the six-marker 28-accession analyses, but in analyses of several markers taken alone, the two representatives of this section appear as a grade adjacent to sect. *Pteroidea*. Additional species of sect. *Herpystichum* are needed to further assess the monophyly of the section and to clarify its precise relationship to other taxa of the Potato clade.

Phylogenetic relationships within *Solanum* sect. *Pteroidea*. — All of the species of *S.* sect. *Pteroidea* for which we were able to sample more than one individual are monophyletic except for *S. mite*. The two accessions of *S. mite* from Ecuador and Peru (3627, 2364) are from lowland Amazonian (220 m) and

mid-elevation Andean (650 m) habitats respectively, whereas the two Bolivian accessions of *S. mite* (2750, 3014) are both from lowland habitats (<300 m). All four of these accessions fit well within the range of morphological variation expected for the species as it is currently circumscribed (Knapp & Helgason, 1997). The unexpected molecular variation in this species, its strongly supported non-monophyly, and the numerous morphological differences between *S. mite* and *S. incurvum* indicate that the widespread *S. mite* may be more complex than we have assumed. Chromosome numbers are unknown for all species of *S.* sect. *Pteroidea*, but based on the clean sequences observed for all markers and because the groupings of *S. mite* fall along geographic lines, we believe that these two groups represent real variation rather than allopolyploidy or hybridization. As a result, it is possible that *S. mite* may contain cryptic species or incomplete lineage sorting in concordance with geographic patterns. A similar case of molecular divergence with minimal morphological divergence was found in diploid wild tomatoes (Peralta & Spooner, 2005) and in the related pepino and its wild relatives (sect. *Basarthrum*; Anderson & al., 2006; Prohens & al., 2006; Blanca & al., 2007); however, subsequent careful examination by the authors revealed morphological variation concordant with the molecular variation. We might expect similar patterns in the even more widespread *S. anceps*, but our sampling is currently limited to accessions from Ecuador, Peru, and Bolivia.

The results presented here are inconclusive regarding the integrity of the two species groups suggested by Knapp & Helgason (1997). Analyses that forced monophyly of the two species groups were not significantly different from unconstrained trees ($P > 0.05$, SH test). However, strong support for the relationship between *S. incurvum* and Bolivian *S. mite* suggests that the groups may not be monophyletic (Figs. 2–3). *Solanum ternatum* is sister to a moderately supported clade that conforms to most members of the *S. mite* species group (Fig. 2; Knapp & Helgason, 1997). The seven species that make up this clade share many morphological characters, including flowers with strongly reflexed corollas at anthesis and plump, ovoid seeds (Knapp & Helgason, 1997). *Solanum savanillense* is not resolved as part of the *S. mite* species group clade, but shares the same set of morphological characters, including strongly reflexed corolla lobes, plump, ovoid seeds, and the sharply pointed, conical fruits typical of most members of the group. The position of *S. savanillense* as separate from other members of the *S. mite* species group, however, is not well supported and in fact, constrained analysis did not support *S. savanillense* as different from the rest of the species of the *S. mite* group. If the placement, however, of *S. savanillense* apart from the other members of the *S. mite* species group is accurate, then the suite of characters mentioned above may have evolved at least twice, or evolved once and was lost in both *S. incurvum* and *S. ternatum*.

Morphological evolution within *Solanum* sect. *Pteroidea*. — One of the goals of this study was to examine the evolution of morphology within a phylogenetic framework. Knapp & Helgason's (1997) cladistic analysis based on morphological characters recovered two subclades within the *S. mite* species group: one containing the simple-leaved species *S. anceps* and *S. angustialatum*, the other containing the compound-leaved *S. chamaepolybotryon*, *S. conicum*, *S. mite*, *S. savanillense*, *S. trizygum*, and *S. uleanum*. Our results are discordant with each of these clades, and according to the phylogenetic hypotheses presented in this study, the patterns of morphological evolution within sect. *Pteroidea* include multiple gains and losses of several notable characters (Fig. 2). Leaf complexity, for example, appears to be a highly variable character and none of the three simple-leaved species, *S. anceps*, *S. angustialatum*, and *S. incurvum*, are each other's closest relatives. Knapp & Helgason (1997) hypothesized a possible ancestor-descendant relationship between *S. anceps* and *S. angustialatum* based on leaf complexity, corolla texture, seed color, and fruit shape, but our analyses do not support such a relationship. In fact, in all cases, the simple-leaved species are most closely related to compound-leaved species. Frequent shifts between simple and compound leaves appear to be a common phenomenon in the evolution of *Solanum* (Anderson & al., 1999), and indeed in many groups of flowering plants (Yi & al., 2004).

Similarly, vininess appears to be quite variable within the section (Fig. 2). Two species of *S.* sect. *Pteroidea*, *S. ternatum* and *S. uleanum*, are truly viny. *Solanum incurvum* appears to be a facultative vine; it can be a free-standing herb, but is sometimes encountered climbing short distances or trailing across the ground. *Solanum incurvum* and *S. ternatum* form the *S. ternatum* species group, but do not emerge as monophyletic in any of our analyses, and neither species is closely related to *S. uleanum*. All members of *Solanum* sections *Herpystichum* and *Anarrhichomenum* are vines, and many members of sect. *Basarthrum* are scandent herbs to shrubs to facultative vines. Thus, there is a tendency toward vininess throughout much of the Potato clade (Child, 1979) and it is possible that vininess is the ancestral condition for *S.* sect. *Pteroidea* and not derived in *S. ternatum* and *S. uleanum*.

Finally, the evolution of the conical fruits characteristic of many species of *S.* sect. *Pteroidea* appears to be similarly complex. Pointed fruits are found in *S. anceps*, *S. angustialatum*, *S. chamaepolybotryon*, *S. conicum*, *S. savanillense*, *S. trizygum*, and *S. uleanum*, whereas *S. incurvum*, *S. mite*, and *S. ternatum* have globose fruits at maturity. Neither conical nor globose fruits characterize monophyletic groups in *S.* sect. *Pteroidea*. The fruits of *S. conicum*, *S. savanillense*, and *S. trizygum* are conical, but are completely filled with seeds (Fig. 1E), in contrast to the apiculate fruits of *S. anceps*, *S. angustialatum*, *S. chamaepolybotryon*, and *S. uleanum* in which the apices are devoid of seeds (Fig. 1D, F). As with leaf complexity, species with these two fruit types do not form monophyletic groups. The discordance between phylogenetic and morphological patterns found in *S.* sect. *Pteroidea* is by no means unique to this group, but rather is a widespread phenomenon reflected even in other groups within the Solanaceae (Bohs & al., 2007).

In summary, *Solanum* sect. *Pteroidea* is strongly supported as monophyletic, but many nodes within the section remain unsupported in all analyses. Highly supported relationships among some species, however, suggest that morphology within sect. *Pteroidea* is highly labile. Frequent origins and losses of traits make it difficult to suggest evolutionary trends for characters unique to *S.* sect. *Pteroidea* such as rugose, apiculate

fruits, and other characters like vininess that are unusual in *Solanum*. The unexpected variation discovered among accessions of *S. mite* deserves more attention, and suggests that the evolutionary history of this species is more complex than initially assumed. Additional accessions from different parts of the ranges of some species, especially *S. trizygum* from Venezuela and the very widespread *S. anceps* are needed to further test the monophyly of these species, and may uncover more variation like that found in *S. mite*. Given the morphological lability apparent in the evolution of this section, and indeed throughout much of *Solanum*, it would not be surprising if a species as widespread and as variable as *S. anceps* were not monophyletic.

■ ACKNOWLEDGEMENTS

The authors thank A. Cano and J. Albán Castillo from the herbarium USM in Lima, Peru for assistance with logistics and permits; R. Vásquez, J. Mateo Miguel, and C. Rojas Tello from the herbarium HOXA in Oxapampa, Peru, and S. Leiva and M. Zapata Cruz of the Universidad Privada Antenor Orrego, Trujillo, Peru, as well as S. Stern and M.P. Moreno for assistance in the field; F.A. Guzmán, S. Stern, and A. Van Wagenen for invaluable assistance in the laboratory; F. Farruggia for assistance with BEST analyses; and D. Spooner, F. Farruggia, and three anonymous reviewers whose comments greatly improved this manuscript. All Bayesian analyses were carried out on the freely available Bioportal (www.bioportal.uio.no). This work was supported by NSF through the PBI: *Solanum* grant, DEB-0316614, to L.B.

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Appendix. Accessions included in this study. Voucher (herbarium acronym), collection locality, GenBank numbers: ITS, GBSSI, *trnT-trnF*, cos1C, cos9B, cos11.

S. anceps Ruiz & Pav., *Bohs 2790a* (UT), Bolivia, GQ221541, GQ221593, GQ221568, GQ221458, GQ221486, GQ221513. ***S. anceps*** Ruiz & Pav., *Bohs 3626* (UT), Ecuador, GQ221542, GQ221594, GQ221569, GQ221459, GQ221487, GQ221514. ***S. anceps*** Ruiz & Pav., *Tepe & Stern 2376* (UT), Peru, GQ221543, GQ221595, GQ221570, GQ221460, GQ221488, GQ221515. ***S. anceps*** Ruiz & Pav., *Tepe 2600* (UT), Ecuador, GQ221544, GQ221596, GQ221571, GQ221461,—, GQ221516. ***S. anceps*** Ruiz & Pav., *Tepe 2608* (UT), Ecuador, GQ221545, GQ221597, GQ221572, GQ221462, GQ221489, GQ221517. ***S. anceps*** Ruiz & Pav., *Tepe 2655* (UT), Ecuador, GQ221546, GQ221598, GQ221573, GQ221463, GQ221490, GQ221518. ***S. angustialatum*** Bitter, *Tepe & Stern 2366* (UT), Peru, GQ221547, GQ221599, GQ221574, GQ221464, GQ221491, GQ221519. ***S. brevifolium*** Dunal, *Bohs 3112* (UT), Ecuador, GQ221562, GQ221614, GQ221589, GQ221480, GQ221507, GQ221535. ***S. bulbocastanum*** Dunal, *Tarn 153* (PTIS), Mexico, GQ221564, DQ169020, DQ180444, GQ221481, GQ221508, GQ221536. ***S. caripense*** Dunal, *Bohs 3149* (UT), Ecuador, GQ221563, GQ221615, GQ221590, GQ221482, GQ221509, GQ221537. ***S. chamaepolybotryon*** Bitter, *Tepe & Stern 2371* (UT), Peru, GQ221548, GQ221600, GQ221575, GQ221465, GQ221492, GQ221520. ***S. conicum*** Ruiz & Pav., *Tepe & Stern 2270* (UT), Peru, GQ221549, GQ221601, GQ221576, GQ221466, GQ221493, GQ221521. ***S. evolvulifolium*** Greenm., *Bohs 2500* (UT), Costa Rica, GQ221565, GQ221616, GQ221591, GQ221483, GQ221510, GQ221538. ***S. incurvum*** Ruiz & Pav., *Tepe & Stern 2294* (UT), Peru, GQ221550, GQ221602, GQ221577, GQ221467, GQ221494, GQ221522. ***S. incurvum*** Ruiz & Pav., *Tepe & Stern 2301* (UT), Peru, GQ221551, GQ221603, GQ221578, GQ221468, GQ221495, GQ221523. ***S. lycopersicum*** L., no voucher, Cultivated: USA, GQ221566, DQ169036, DQ180450, GQ221484, GQ221511, GQ221539. ***S. mite*** Ruiz & Pav., *Bohs 2750* (UT), Bolivia, GQ221552, GQ221604, GQ221579, GQ221469, GQ221496, GQ221524. ***S. mite*** Ruiz & Pav., *Bohs 3014* (UT), Bolivia, GQ221553, GQ221605, GQ221580, GQ221470, GQ221497, GQ221525. ***S. mite*** Ruiz & Pav., *Bohs 3627* (UT), Ecuador, GQ221554, GQ221606, GQ221581, GQ221471, GQ221499, GQ221527. ***S. mite*** Ruiz & Pav., *Tepe & Stern 2364* (UT), Peru, GQ221555, GQ221607, GQ221582, GQ221472, GQ221498, GQ221526. ***S. phaseoloides*** Pol., *Bohs 2485* (UT), Costa Rica, GQ221567, GQ221617, GQ221592, GQ221485, GQ221512, GQ221540. ***S. savanillense*** Bitter, *Bohs 3444* (UT), Ecuador, GQ221556, GQ221608, GQ221583, GQ221473, GQ221500, GQ221528. ***S. ternatum*** Ruiz & Pav., *Bohs 3395* (UT), Ecuador, GQ221557, GQ221609, GQ221584, GQ221474, GQ221501, GQ221529. ***S. ternatum*** Ruiz & Pav., *Tepe & Stern 2320* (UT), Peru, –, GQ221610, GQ221585, GQ221475, GQ221502, GQ221530. ***S. trizygum*** Bitter, *Bohs 3509* (UT), Costa Rica, GQ221558, GQ221611, GQ221586, GQ221476, GQ221503, GQ221531. ***S. trizygum*** Bitter, *Moran 7678* (UT), Costa Rica, GQ221559, GQ221612, GQ221587, GQ221477, GQ221504, GQ221532. ***S. uleanum*** Bitter, *Bohs 2720* (UT), Cultivated (Seeds: *D'Arcy, s.n.*), GQ221560, DQ169052, DQ180472, GQ221478, GQ221505, GQ221533. ***S. uleanum*** Bitter, *Bohs 3628* (UT), Ecuador, GQ221561, GQ221613, GQ221588, GQ221479, GQ221506, GQ221534.