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Phylogenetic Relationships of *Solanum* Series *Conicibaccata* and Related Species in *Solanum* Section *Petota* Inferred from Five Conserved Ortholog Sequences

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Abstract—*Solanum* series *Conicibaccata* is the second largest series in section *Petota*, containing 40 species widely distributed from southern Mexico to central Bolivia. It contains diploids ($2n = 2x = 24$), tetraploids ($2n = 4x = 48$) and hexaploids ($2n = 6x = 72$), and the limited number of species examined have been shown to be allopolyploids. Previous morphological and molecular studies using plastid DNA failed to discriminate clear species boundaries. Conserved orthologous nuclear DNA sequences (COSII) were used to compare the relationships among 72 accessions from 22 species from series *Conicibaccata* and 42 additional accessions from related series. The results supported previous studies showing the diploid members of series *Conicibaccata* to be related to other South American "clade 4" species, and showed all of the polyploids to be allopolyploids among members of clade 4 and other South American species of "clade 3" (series *Piurana* and related species). Low bootstrap support values and morphological similarity suggest recent origins and the need for a reduction in number of recognized species in series *Conicibaccata*.

Keywords—Conserved orthologous sequences, COSII, *Solanum* section *Petota*, *Solanum* series *Conicibaccata*, wild potatoes.

Solanum L. section *Petota* Dumort., the potato and its wild relatives, are widely distributed in the Americas from the southwestern U. S. A. to Chile, Argentina, and Uruguay. The taxonomy of section *Petota* is complicated by sexual compatibility among many species, introgression, interspecific hybridization, auto- and allopolyploidy, a mixture of sexual and asexual reproduction, possible recent species divergence, phenotypic plasticity, and consequent morphological similarity and difficulty in defining and distinguishing species and series (Spooner and van den Berg 1992; Spooner 2009). These complicating biological factors have led to differences among taxonomic treatments by different authors. The latest taxonomic estimate in section *Petota* is about 100 wild species and four cultivated species (Spooner et al. 2009). This differs fundamentally from a previous estimate of 217 wild species and seven cultivated species (Hawkes 1990).

Series *Conicibaccata* Bitter is the second largest series in section *Petota*, after series *Tuberosa* (Rydb.) Hawkes, and these are the only two series distributed in both North and Central America and in South America. Series *Conicibaccata* is taxonomically difficult, and has been thought to contain 40 species of diploids ($2n = 2x = 24$), tetraploids ($2n = 4x = 48$) and hexaploids ($2n = 6x = 72$) (Hawkes 1990). Previous morphological evaluations of the series (Castillo and Spooner 1997; Fajardo et al. 2008) documented the difficulty in the use of taxonomic keys because of high overlap of the traits distinguishing the species, the lack of comprehensive evaluations of all the species, and the need to reevaluate the circumscription of the series. As outlined in previous papers (e.g., Spooner and Castillo 1997; Rodríguez and Spooner 2009) the traditional series classifications of have received poor support, and our use of the term series follows Hawkes (1990).

Plastid DNA restriction site studies (Spooner and Sytsma 1992; Castillo and Spooner 1997; Rodríguez and Spooner 1997; Spooner and Castillo 1997) defined four clades in section *Petota*: clade 1 contains diploid species from North and Central America (except *S. bulbocastanum* Dunal, *S. cardiophyllum* Lindl. and *S. verrucosum* Schltdl.), clade 2 contains the North and Central American diploid species *S. bulbocastanum* and *S. cardiophyllum*, clade 3 contains series *Piurana* Hawkes and some species from series *Conicibaccata* (*S. chomatophilum* Bitter, *S. contumazaense* Ochoa, *S. irosinum* Ochoa and *S. paucijugum* Bitter), *Megistacroloba* Cárdenas et Hawkes, *Tuberosa*, and *Yungasensa* Correll, and clade 4 contains the Mexican diploid species *S. verrucosum*, the remaining South American species, and the North and Central American polyploids. There was poor resolution within these clades, especially clade 4 which contained most of the species of ser. *Conicibaccata*, possibly caused by slow divergence of the plastid DNA (Sang 2002).

Jiménez et al. (2008) explored the utility of AFLPs to define species boundaries within series *Conicibaccata*, but many species were intermixed on the same clade. Spooner et al. (2008) and Rodríguez and Spooner (2009) used the single-copy nuclear DNA sequences GBSSI and nitrate reductase respectively, and documented allopolyploid origins of four polyploid members of series *Conicibaccata* (*S. agrimonifolium* Rydb., *S. colombianum* Dunal, *S. longiconicum* Bitter and *S. moscopanum* Hawkes). However, Spooner et al. (2008) and Rodríguez and Spooner (2009) did not examine enough species or accessions within species to see if all the species were allopolyploid, or if the species boundaries were distinct. The present study examines DNA sequences of conserved orthologous sequences (COSII) of 22 of the 40 species of series *Conicibaccata* sensu Hawkes (1990) that were available in germplasm collections, and species from other series to address allopolyploid origins.

Materials and Methods

Plant Material—Total genomic DNA was isolated from young leaves of single plants of 72 accessions from 22 species of series *Conicibaccata* (Appendix 1) according to Castillo and Spooner (1997), using standard protocols (Ghislain 1999). Identifications of the earlier collected accessions were provided by multiple visits to the CIP and US genebanks by Jack Hawkes and Carlos Ochoa, and the later ones by Spooner and collaborators. Vouchers are deposited in the herbaria of the International Potato Center at Lima, Peru (no formal herbarium acronym but we refer to as CIP) and at the United States Potato Introduction Station Herbarium (PTIS). DNA quantity was estimated by comparison with the 11,490 bp fragment of 1 µg of Lambda DNA (Gibco-BRL, Gaithersburg, Md.) digested with *Pst*I and subjected to electrophoresis on a 1% agarose gel, stained with ethidium bromide. Fifteen accessions from 14 species of series *Piurana*, 28 representative accessions from clade 4, and the North and Central American diploids (clades 1 + 2), according to previous plastid DNA (Spooner and Sytsma 1992; Castillo and Spooner 1997; Rodríguez

and Spooner 1997; Spooner and Castillo 1997) and DNA sequence studies (Spooner et al. 2008; Rodríguez and Spooner 2009; Ames and Spooner 2010) were included in the analysis. The results of the plastid and nuclear DNA studies are similar except that the nuclear data unite plastid clades 1 and 2 (hence we refer to this clade as 1 + 2). Four additional accessions from section *Etuberosum* A. Child (*S. etuberosum* Lindl. and *S. fernandezianum* Phil.) were included as outgroups, following Spooner et al. (1993, 2008) and Rodríguez et al. (2009), for a total of 119 accessions.

DNA Extraction and PCR Sequencing—Five pairs of COSII primers chosen to represent maximum polymorphism and concordance among results of section *Petota* were used, based on Rodríguez et al. (2009), who initially chose these primers due to 1) 70% or more intron content, 2) sequence length of 700–1,300 bp, and 3) good genomic coverage. The primer pairs (forward and reverse, respectively) used in the analyses were: COSII-3 (5′-TCAACAAGAGTACACGGTTTGAAGAC-3′ and 5′-TTGCTCTAGCCCTGGCCCTAAC-3′), COSII-9 (5′-TGCAGCTTTGCTTTATGATGCC-3′ and 5′-AAAGGCTTGGCCGTAGCTTGC-3′), COSII-11 (5′-TTCTCTTTCCCTTATCTGCAACAC-3′ and 5′-TCCTTCAATCATGTACTTAGAGACTTC-3′), COSII-1C (5′-AGGTGCTTTCTTGTTTCTTCTTTC-3′ and 5′-AGAGCATATCACGATACTTGGTGTG-3′) and COSII-3C (5′-TGATCTAAAATTGCCTGGTTTTG-3′ and 5′-AATAGCCCTCAAGGACCATGTGG-3′).

Methods used by Rodríguez et al. (2009) were followed for DNA extraction, PCR conditions, cloning, and sequencing. For DNA sequencing of the polyploid species, DNA bands from all 37 accessions were cut and purified from the gel using a Zymoclean Gel DNA Recovery Kit (Zymo Research, Orange, California). To ensure 95% confidence that all possible sequences could be selected and sequenced during the sampling, five transformed colonies were chosen for selected diploid accessions that provided two peaks from direct sequencing, ten for the tetraploids, and 20 for the hexaploids.

DNA sequences were edited using Staden package version 1.6.0 (Staden 1996), and sequence alignments were conducted using ClustalX version 2.0 (Thompson et al. 1997) using the default parameters of a gap opening penalty of 10, a gap extension penalty of 0.20, and a delay divergent sequence percentage of 30%. Subsequent minor gap alignments were done by eye using MacClade version 4.08 (Maddison and Maddison 2000). Potential PCR recombinants were identified by visual inspection using MacClade 4.08 (Maddison and Maddison 2000) and deleted from the analyses.

Phylogenetic Analyses—Two datasets were examined: 1) a dataset containing only diploid species, 2) the complete dataset containing all diploid and polyploid species. Both datasets included the outgroups and species outside series *Conicibaccata*, and were used to reconstruct the phylogenetic relationships. PAUP* version 4.0b8 (Swofford 2002) was used for maximum parsimony (MP) analyses. Gaps were scored using SeqState version 1.4 (Müller 2005) by the simple gap scoring method (Simmons and Ochoterena 2000). All characters were treated as unordered and weighted equally (Fitch 1971). The most parsimonious trees were found by heuristic searches under Fitch criteria (Farris 1970) and equal weight for all characters by generating 100,000 replicates and one tree held for each replicate, a random order entry and tree-bisection reconnection (TBR) as the branch-swapping method, retaining all most parsimonious trees. Support values for individual clades were estimated with bootstrap analyses (Felsenstein 1985) using 1,000 replicates, using the same search criteria as above.

Maximum likelihood (ML) analyses for both the diploid and polyploid datasets were conducted after selecting the best fit evolutionary model for the sequence data with Modeltest v. 3.7 (Posada and Crandall 1998). Maximum likelihood analyses were conducted using RAxML v. 7.0.4 (Stamatakis et al. 2008) in the Cyberinfrastructure for Phylogenetic Research (CIPRes) cluster at San Diego Supercomputer Center (http://www.phylo.org) with 1,000 bootstrap runs.

Templeton Tests—Templeton tests were used to assess: 1) whether each of the diploid species recognized by Hawkes (1990) was supported as monophyletic, 2) whether the diploid accessions of ser. *Conicibaccata* as a group were supported as monophyletic. We performed these tests by calculating the nonparametric Wilcoxon signed rank test (Templeton 1983). The analyses were run in PAUP* version 4.0b8 (Swofford 2002), enforcing topological constraints in a heuristic search using TBR and 100 random addition replicates, saving no more than 10 trees per replicate.

Results

COSII Sequence Data—A total of 535 sequences were examined (five clones from 17 diploid accessions showing two peaks, ten clones from all 29 tetraploid accessions, and 20 clones from all eight hexaploid accessions) for each of the five COSII, for a total of 2,675 sequences. Primary sequence types of the polyploids were identified for a given accession and we summarized minor differences of a reduced number of clones within these types by using ambiguity codes. The alignment was 5,158 bp long, and individual COSII length sequences ranged from 821–848 bp for COSII-1C, 454–457 bp for COSII-3, 812–818 bp for COSII-3C, 1,769–2,389 bp for COSII-9, and 633–646 bp for COSII-11. The aligned matrix is available in TreeBASE (study number S10707).

Heuristic searches under maximum parsimony with and without gap scores were performed. The comparison between the two showed similar topologies and slightly higher bootstrap values in the analyses performed without gap scores, and further parsimony analyses were run without gaps, which may have contributed additional homoplasy to the results. The number of constant, variable, and parsimony informative characters for each COSII for both datasets is summarized in Table 1. Only two COSII (COSII-3 and COSII-11) were less than the 70% intron content sought by Rodríguez et al. (2009).

Table 1. Proportion of variable, constant, parsimony uninformative (PU) and parsimony informative (PI) characters from each COSII marker and total database with their respective percentages calculated from the total character length. [1]Percentage calculated from intron length. [2]Percentage calculated from the number of exon aligned characters. [3]Dataset containing the outgroups, members from clade 1 + 2, 3 and all members from series *Conicibaccata*. [4]Dataset containing the outgroups, members from clades 1 + 2, 3 and only diploid species from ser. *Conicibaccata*.

	Complete dataset[3]						Reduced dataset[4]					
	COSII-3	COSII-9	COSII-11	COSII-1C	COSII-3C	**Total**	COSII-3	COSII-9	COSII-11	COSII-1C	COSII-3C	**Total**
Total characters	457	2389	646	848	818	**5158**	457	2202	646	847	818	**4970**
Constant characters	367 80.30%	1931 80.80%	442 68.40%	615 75.10%	575 70.20%	**3930 76.20%**	389 85.12%	1805 81.97%	469 72.60%	658 77.69%	625 76.41%	**3946 79.40%**
Variable PU characters	49 10.70%	213 8.90%	83 12.80%	82 9.60%	100 12.20%	**527 10.20%**	34 7.44%	186 8.45%	74 11.46%	76 8.97%	69 8.44%	**439 8.83%**
PI characters	41 8.90%	245 10.20%	121 18.70%	151 17.80%	143 17.40%	**701 13.60%**	34 7.44%	211 9.582%	103 15.94%	113 13.34%	124 15.16%	**585 11.77%**
Intron aligned characters	217 47.50%	2338 97.80%	413 63.90%	677 79.80%	739 90.30%	**4384 85.00%**	217 47.48%	2151 97.68%	413 63.93%	676 79.81%	739 90.34%	**4196 84.43%**
Intron PI characters[1]	32 14.70%	240 10.20%	98 23.70%	134 19.80%	138 18.70%	**642 14.60%**	27 12.44%	206 9.58%	84 20.34%	101 14.94%	120 16.24%	**538 12.82%**
Exon aligned characters	240 52.50%	51 2.20%	233 36.10%	171 20.20%	79 6.70%	**774 15.00%**	240 52.52%	51 2.32%	233 36.07%	171 20.19%	79 9.66%	**774 15.57%**
Exons PI characters[2]	9 3.70%	5 9.80%	23 9.90%	17 9.90%	5 6.30%	**59 7.60%**	7 2.92%	5 9.80%	19 8.15%	12 7.02%	4 5.06%	**47 6.07%**

The ILD test for each pairwise comparison of the five COSII phylogenies showed incongruent topologies ($p \leq 0.001$) with the reduced and complete datasets, and with a new data set including the outgroup species and only the diploid species from series *Conicibaccata* without members and placeholder accessions from clades 1 + 2, 3 and 4. However, combining the five COSII regions resulted in a better-resolved tree with higher branch support for clades (Fig. 1) than any of the single COSII partitions. The incongruence among the COSII independent phylogenies as shown by the ILD test might be a result of widespread reticulation, and/or multiple independent origins of the allopolyploids (Symonds et al. 2010), preventing them from becoming sexually distinct lineages (Linder and Rieseberg 2004; Soltis and Soltis 2009).

FIG. 1. Maximum likelihood phylogram of *Solanum* series *Conicibaccata*. The values above the branches are bootstrap values equal or higher than 50% obtained by maximum likelihood. The accessions are identified by their PI or CIP number (see Appendix 1). The suffix corresponds to the allele coded for that accession. The accessions coded in green correspond to the diploid members of series *Piurana* (clade 3), in red to diploid members of series *Conicibaccata* (clade 4) and in blue the amplicons of the polyploid members of series *Conicibaccata* partitioned in both clades 3 and 4. Branches of other series (black) with support below 70% were collapsed.

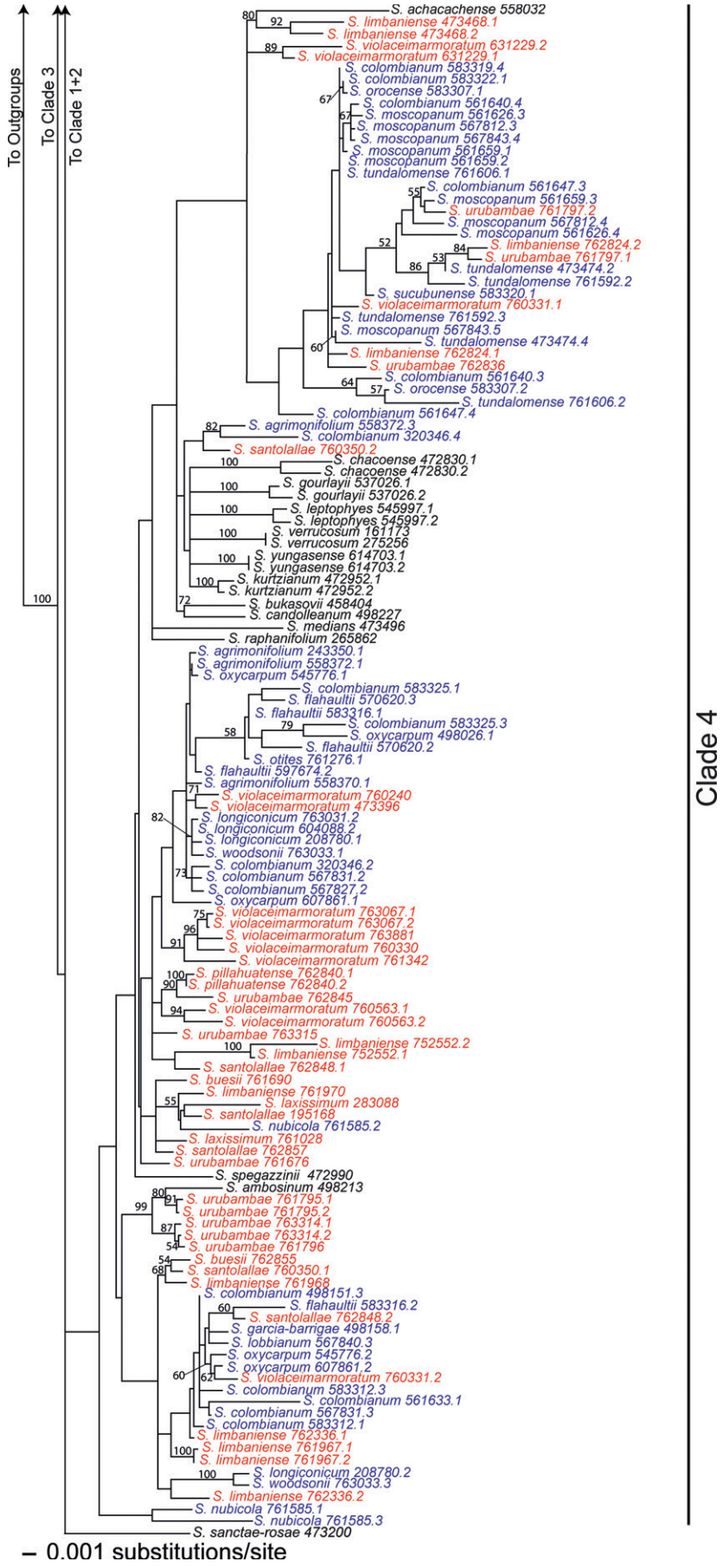

FIG. 1. Continued.

***Phylogenetic Analyses*—**The MP heuristic search of the reduced dataset found 21 most parsimonious trees of 2,081 steps, with consistency index (CI) of 0.561, rescaled consistency index (RC) of 0.418, and retention index (RI) of 0.745. The consensus tree showed similar clade structure compared to the ML phylogram and no significant differences at the species and accession levels. The best model fit selected by the Akaike Information Criterion (AIC) for the dataset containing only the diploid members from the series was GTR + Γ + I, lnL = -21,770.0332, Γ = 0.7615, I = 0.5246 with base frequencies A = 0.2673, C = 0.1917, G = 0.1851 and T = 0.3559, and substitution rate matrix [A↔C] = 0.8012, [A↔G] = 3.6341, [A↔T] = 1.0604, [C↔G] = 0.7901, [C↔T] = 2.6240, [G↔T] = 1.0000.

After incorporating the polyploid species from series *Conicibaccata* into the original dataset, the MP heuristic search found six most parsimonious trees with a total length of 3,403, CI = 0.409, RC = 0.333 and RI = 0.813. The trees showed a resolved structure of clade 1 + 2, clade 3 (now including amplicons from members of series *Conicibaccata*) and clade 4 (containing also the corresponding amplicons from polyploid members from series *Conicibaccata*). Low bootstrap values (< 50) were obtained for the nodes supporting clades 3 and 4, while the bootstrap support value for clade 1 + 2 was 100. All diploid members from series *Conicibaccata* were part of clade 4 containing diverse placeholder representative members of this clade, except for *S. trinitense* which resolved in clade 3.

For the ML analysis, the best fit model selected by the AIC obtained with Modeltest for the combined sequence dataset was GTR + Γ + I, lnL = -30,655.9062, Γ = 0.4410, I = 0.5049 with base frequencies A = 0.2621, C = 0.1943, G = 0.1890 and T = 0.3547, and substitution rate matrix [A↔C] = 0.9580, [A↔G] = 3.6615, [A↔T] = 1.2382, [C↔G] = 0.7945, [C↔T] = 3.0122, [G↔T] = 1.0000. The main difference from the topology of the ML phylogram versus the most parsimonious trees was the relationship of the clade 1 + 2, where in ML it appears as sister clade of clade 4 (Fig. 1). *Solanum trinitense* Ochoa remained as part of clade 3.

An examination of relationships within clades 3 and 4 showed no species-specific clades for any of the species from series *Conicibaccata*. Also, no geographical pattern was found in any clade or subclades. The amplicons from the polyploid species resolved in both clades 3 and 4 as found in previous studies by Spooner et al. (2008) and Rodríguez and Spooner (2009), and clade 4 forms two subclades, one of them containing the members from the *Solanum brevicaule* Bitter complex, a group of similar species that are the progenitors of the cultivated potato. The species from series *Piurana* are dispersed in clade 3 (Spooner et al. 2005).

The MP and ML analyses for the reduced diploid dataset (not shown) showed low bootstrap values (57%) for clade 4, 77% and 87% for clade 3, and 100% for clade 1 + 2. The entire diploid and polyploid dataset showed low bootstrap values <50% for clade 4, < 50% for clade 3, and 100% for clade 1 + 2. No specific accessions or groups of accessions were identified as causing these low clade-specific bootstrap values. No species-specific clades were found, and all diploid members of ser. *Conicibaccata* resolve inside clade 4 with the exception of *S. trinitense* which resolved in clade 3. All accessions of all 12 polyploid species of series *Conicibaccata* contained alleles in both clades 3 and 4, concordant with the previous results of Spooner et al. (2008) and Rodríguez and Spooner (2009).

The Templeton tests for 1) monophyly of the diploid species of ser. *Conicibaccata* and 2) whether the diploid species of series *Conicibaccata* were monophyletic as a group were nonsignificant because of so little structure in clade 4.

Discussion

***Phylogenetic Analyses*—**The combined morphological (Fajardo et al. 2008) and present COSII results make it clear that wild potato species from series *Conicibaccata* are a difficult group, as evidenced by morphological similarity among its constituent traditionally recognized species and reticulate evolution of the polyploids. Wendel and Doyle (1998) pointed out that incongruence of different data sets is common and that relying on a single dataset may lead to misleading inferences. Even though the debate continues on merging or combining datasets showing different gene histories, our objective was to try to maximize the number of variable sites to obtain sufficient phylogenetic signal to discriminate among these similar species. As mentioned above, the lack of congruence among different COSII markers as assessed by the ILD test might be a result of widespread reticulation, and/or multiple independent origins of the allopolyploids.

***Diploid Species*—**The reduced dataset excluding the polyploid species from series *Conicibaccata* showed an overall structure similar to that previously reported by Spooner et al. (2008) and Rodríguez and Spooner (2009) with other nuclear DNA markers. That is, the traditionally recognized diploid species resolved inside clade 4, 1) were not monophyletic, 2) the clades had no correspondence with geographical distributions, 3) most of the clades had low bootstrap support, 4) the clades failed to group morphologically similar traditionally recognized species, for example, *S. laxissimum* Bitter and *S. santolallae* Vargas, or *S. urubambae* Juz. and *S. violaceimarmoratum* Bitter. The only traditionally recognized diploid member of ser. *Conicibaccata* not falling in clade 4 is *S. trinitense*, a member of clade 3 and clearly misplaced in the series based on a wider examination of clade 3 species by Ames and Spooner (2010). Our COSII results concur with those of Spooner and Castillo (1997), Castillo and Spooner (1997), and Ames and Spooner (2010) in showing the need to redefine ser. *Conicibaccata* by moving some species into a redefined and expanded ser. *Piurana* (clade 3).

***Polyploid Species*—**The results are concordant with prior results in 1) supporting allopolyploid origins of all polyploid members of ser. *Conicibaccata* (Spooner et al. 2008; Rodríguez and Spooner 2009), 2) as with the diploid species, supporting the need to reduce the number of species, 3) failing to produce cladograms with high bootstrap support for clades 3 and 4 or for grouping many traditionally recognized species within these clades (Castillo and Spooner 1997; Spooner and Castillo 1997; Jacobs et al. 2008). The inconsistency between the ML phylogram with MP results, showing clades 1 + 2 and clade 4 as sister clades, rather than clades 3 and 4 as sister, is not significant in our dataset as it is due to soft incongruence and low bootstrap support for these clades. Possible hybridization inside series *Conicibaccata* and with related series (as *Piurana*), is reflected by the lack of resolution of clades 4 and 3.

Wild potato species in series *Conicibaccata* are clearly overdescribed, with few if any species-specific morphological traits. Only a few species such as *S. flahaultii* Bitter (4*x*), *S. longiconicum* Bitter (4*x*), and *S. trinitense* (2*x*) were found to be clearly distinct by morphometric studies (Fajardo et al. 2008). *Solanum trinitense* clearly should be excluded from ser. *Conicibaccata* and included in series *Piurana*, and this is the

only 2*x* (1EBN) reported species within all the 2*x* (2EBN) species from series *Conicibaccata* (Hawkes 1990). Entirely on the basis of empirical data, *Solanum* species have been assigned EBN numbers based on their ability to hybridize with each other (Hanneman 1994). Barring other crossing barriers, successful hybridization is expected when male and female gametes have matching EBN values, regardless of ploidy. Ploidy (EBN) combinations in potato include 6*x* (4EBN), 4*x* (4EBN), 4*x* (2EBN), 2*x* (2EBN), and 2*x* (1EBN). All of these levels occur in ser. *Conicibaccata* except 4*x* (4EBN) and 2*x* (1EBN) with the exclusion of *S. trinitense* (Castillo and Spooner 1997). Even the morphologically most distinctive species within ser. *Conicibaccata*, *S. flahaultii* (4x) and *S. longiconicum* (4x) (Fajardo et al. 2008) did not form species-specific clades. In the case of *S. longiconicum* their amplicons resolved with *S. woodsonii* (4x) Correll (also a tetraploid), while the different amplification products of *S. flahaultii* resolved with the diploid *S. santolallae* (2x) and the polyploids *S. colombianum* (4x) and *S. otites* Dunal (4x). The relationship between *S. longiconicum* and *S. woodsonii* may be explained by their overlapping geographical distributions and possible hybridization, similar to the relationship of *S. colombianum* and *S. flahaultii*, but overlapping ranges do not explain the relationship of *S. otites* and *S. santolallae*.

For the rest of the species, no clear species-specific clades were found in the molecular analyses, corroborating the morphological data (Fajardo et al. 2008) showing few species-specific groups. Although five COSII markers produced a cladogram with well-resolved branches in the study of Rodríguez et al. (2009), that study used only a few morphologically distinctive representatives from subgenus *Potatoe* (G. Don) D'Arcy (Rodríguez and Spooner 2009), not using the many more morphologically similar (some likely conspecific) species examined here. Morphological studies of series *Conicibaccata* showed the need to reduce the number of species, but with some species clearly defined. As outlined by Spooner (2009), the taxonomy of section *Petota* is complicated by sexual compatibility among many species, introgression, interspecific hybridization, auto- and allopolyploidy, a mixture of sexual and asexual reproduction, possible recent species divergence, phenotypic plasticity, and consequent morphological similarity and difficulty in defining and distinguishing species and series. The diploid and polyploid members of series *Conicibaccata* seem to be representative of section *Petota* as a whole, indicating the need for great reduction of species.

Decisions on species boundaries will be formalized, to include proper typifications, in a taxonomic monograph of section *Petota* in revision for southern South America and in preparation for northern South America. The decisions will consider data from evolutionary history (this study), morphological phenetics (Fajardo et al. 2008), and study of herbarium specimens, including types. We likely will follow synonymy currently listed in an informal way on the Solanaceae Source website: www.nhm.ac.uk/solanaceaesource. As with other complicated groups experiencing the possible recent and rapid evolution and possible hybridization common in section *Petota* (Spooner 2009), there is no rigid formula for such decisions.

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Literature Cited

Ames, M. and D. M. Spooner. 2010. Phylogeny of *Solanum* series *Piurana* and related species in *Solanum* section *Petota* based on five conserved ortholog sequences. *Taxon* 59: 1091–1101 + 4-page foldout.

Castillo, R. and D. M. Spooner. 1997. Phylogenetic relationships of wild potatoes, *Solanum* series *Conicibaccata* (sect. *Petota*). *Systematic Botany* 22: 45–83.

Fajardo, D., R. Castillo, A. Salas, and D. M. Spooner. 2008. A morphometric study of species boundaries of the wild potato *Solanum* series *Conicibaccata*: a replicated field trial in Andean Peru. *Systematic Botany* 33: 183–192.

Farris, J. 1970. Methods for computing Wagner trees. *Systematic Zoology* 19: 83–92.

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

Fitch, W. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* 20: 406–416.

Ghislain, M. 1999. *Molecular biology laboratory protocols: plant genotyping training manual*. Lima: International Potato Center (CIP).

Hanneman, R. E. Jr. 1994. Assignment of endosperm balance numbers to the tuber-bearing solanums and their close non-tuber-bearing relatives. *Euphytica* 74: 19–25.

Hawkes, J. G. 1990. *The potato: evolution, biodiversity and genetic resources*. London: Belhaven Press.

Hijmans, R., T. Gavrilenko, S. Stephenson, J. Bamberg, A. Salas, and D. M. Spooner. 2007. Geographic and environmental range expansion through polyploidy in wild potatoes (*Solanum* section *Petota*). *Global Ecology and Biogeography* 16: 485–495.

Jacobs, M., R. van den Berg, V. Vleeshouwers, M. Visser, R. Mank, M. Sengers, R. Hoekstra, and B. Vosman. 2008. AFLP analysis reveals a lack of phylogenetic structure within *Solanum* section *Petota*. *BMC Evolutionary Biology* 8: 145–157.

Jiménez, J. P., A. Brenes, D. Fajardo, A. Salas, and D. M. Spooner. 2008. The use and limits of AFLP data in the taxonomy of polyploid wild potato species in *Solanum* series *Conicibaccata*. *Conservation Genetics* 9: 381–387.

Linder, C. R. and L. H. Rieseberg. 2004. Reconstructing patterns of reticulate evolution in plants. *American Journal of Botany* 91: 1700–1708.

Maddison, D. and W. Maddison. 2000. MacClade 4: Analysis of phylogeny and character evolution. Arizona: University of Arizona.

Müller, K. 2005. SeqState - primer design and sequence statistics for phylogenetic DNA data sets. *Applied Bioinformatics* 4: 65–69.

Posada, D. and K. Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Rodríguez, A. and D. Spooner. 1997. Chloroplast DNA analysis of *Solanum bulbocastanum* and *S. cardiophyllum*, and evidence for the distinctiveness of *S. cardiophyllum* subsp. *ehrenbergii* (sect. *Petota*). *Systematic Botany* 22: 31–43.

Rodríguez, F. and D. M. Spooner. 2009. Nitrate reductase phylogeny of potato (*Solanum* sect. *Petota*) genomes with emphasis on the origins of the polyploid species. *Systematic Botany* 34: 207–219.

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

Sang, T. 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Reviews in Biochemistry and Molecular Biology* 37: 121–147.

Simmons, M. and H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.

Soltis, P. S. and D. E. Soltis. 2009. The role of hybridization in plant speciation. *Annual Review of Plant Biology* 60: 561–588.

Spooner, D. M. 2009. DNA barcoding will frequently fail in complicated groups: an example in wild potatoes. *American Journal of Botany* 96: 1177–1189.

Spooner, D. M., M. Ames, D. Fajardo, and F. Rodríguez. 2009. Species boundaries and interrelationships of *Solanum* sect. *Petota* (wild and cultivated potatoes) are drastically altered as a result of PBI-funded research. Botany and Mycology 2009 Meeting Abstracts http://2009.botanyconference.org/engine/search/index.php?func=detail&aid=24.

Spooner, D. and R. Castillo T. 1997. Reexamination of series relationships of South American wild potatoes (Solanaceae: *Solanum* sect. *Petota*): evidence from chloroplast DNA restriction site variation. *American Journal of Botany* 84: 671–685.

Spooner, D. and K. J. Sytsma. 1992. Reexamination of series relationships of Mexican and Central American wild potatoes (*Solanum* sect. *Petota*): evidence from chloroplast DNA restriction site variation. *Systematic Botany* 17: 432–448.

Spooner, D. and R. G. van den Berg. 1992. Species limits and hypotheses of hybridization of *Solanum berthaultii* Hawkes and *S. tarijense* Hawkes: morphological data. *Taxon* 41: 685–700.

Spooner, D., G. J. Anderson, and R. K. Jansen. 1993. Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes, and pepinos (Solanaceae). *American Journal of Botany* 80: 676–688.

Spooner, D., K. McLean, G. Ramsay, R. Waugh, and G. J. Bryan. 2005. A single domestication for potato based on multilocus AFLP genotyping. *Proceedings of the National Academy of Sciences USA* 102: 14694–14699.

Spooner, D., F. Rodríguez, Z. Polgar, H. E. Ballard Jr., and S. H. Jansky. 2008. Genomic origins of potato polyploids: GBSSI gene sequencing data. *The Plant Genome, a supplement to Crop Science* 48: S27–S36.

Staden, R. 1996. The Staden sequence analysis package. *Molecular Biotechnology* 5: 233–241.

Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* 75: 758–771.

Swofford, D. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0b3a PPC. Sunderland: Sinauer Associates.

Symonds, V. V., P. S. Soltis, and D. E. Soltis. 2010. Dynamics of polyploid formation in *Tragopogon* (Asteraceae): recurrent formation, gene flow, and population structure. *Evolution* 64: 1984–2003.

Templeton, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* 37: 221–244.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The CLUSTALX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 5: 4876–4882.

Wendel, J. F. and J. J. Doyle. 1998. Phylogenetic incongruence: window into genome history and molecular evolution. Pp. 265–296 in *Molecular Systematics of Plants II*. ed. D. Soltis, P. Soltis and J. Doyle. New York: Springer-Verlag.

Appendix 1. *Solanum* series *Conicibaccata* species used for analyses. See Hijmans et al. (2007) for references documenting individual chromosome counts. Accessions with the prefix "CIP" are from the International Potato Center, and those with the prefix "PI" (Plant Introduction) are from the US Potato Genebank in Sturgeon Bay, Wisconsin. Generalized map localities as illustrated in Supplementary Figs. 1-3 of Fajardo et al. (2008) deposited on the *Systematic Botany* Supplementary data website at: http://www.aspt.net/publications/sysbot/supdata_systbot.php, species name, collector and collector number, USDA Plant Introduction [PI] number, country, first-level administrative subdivision and NCBI numbers (for COSII-1C; COSII-3; COSII-3C; COSII-9 and COSII-11). More complete locality data are provided in Fajardo et al. (2008).

Map Locality 1: *S. oxycarpum, Tarn et al. 182,* PI 498026, Mexico, Puebla (HM747404, HM747405, HM747406, HM747407; HM747543, HM747544; HM747672, HM747673; HM747782, HM747783, HM747784; HM747891). Map Locality 2: *S. oxycarpum, Tarn et al. 272,* PI 545776, Mexico, Oaxaca (HM747408, HM747409; HM747545, HM747546; HM747674, HM747675; HM747785, HM747786, HM747787; HM747892, HM747893). Map Locality 3: *S. oxycarpum, Rivera-Peña et al. 960,* PI 607861, Mexico, Chiapas (HM747410, HM747411; HM747547, HM747548; HM747676, HM747677; HM747788, HM747789, HM747790; HM747894, HM747895). Map Locality 4: *S. agrimonifolium, Spooner et al. 4227,* PI 558372, Mexico, Chiapas (HM747315, HM747316, HM747317; HM747460, HM747461; HM747589; HM747724, HM747725; HM747824, HM747825). Map Locality 5: *S. agrimonifolium, Spooner et al. 4208,* PI 558370, Mexico, Chiapas (HM747313, HM747314; HM747457, HM747458, HM747459; HM747588; HM747722, HM747723; HM747822, HM747823). Map Locality 6: *S. agrimonifolium, Graham 145,* PI 243350, Guatemala, Quezaltenango (HM747309, HM747310, HM747311, HM747312; HM747455, HM747456; HM747586, HM747587; HM747720, HM747721; HM747820, HM747821). Map Locality 7: *S. longiconicum, Hope s. n.,* PI 208780, Costa Rica, Alajuela (HM747369, HM747370; HM747510, HM747511, HM747512; HM747636, HM747637, HM747638; HM747763, HM747764, HM747765; HM747864, HM747865). Map Locality 9: *S. longiconicum, Spooner et al. 7103,* PI 604088, Costa Rica, Cartago (HM747367, HM747368; HM747508, HM747509; HM747634, HM747635; HM747761, HM747762; HM747862, HM747863). Map Locality 10: *S. longiconicum, Spooner et al. 7411*, CIP 763031, Panama, Chiriquí (HM747365, HM747366; HM747506, HM747507; HM747632, HM747633; HM747759, HM747760; HM747860, HM747861); *S. woodsonii,* CIP 473474, Panama (HM747453, HM747454; HM747583, HM747584, HM747585; HM747717, HM747718, HM747719; HM747818, HM747819; HM747934, HM747935). Map Locality 11: *S. colombianum, Spooner et al. 6319,* PI 583325, Venezuela, Táchira (HM747331, HM747332, HM747333; HM747476, HM747477; HM747604, HM747605; HM747732, HM747733, HM747734; HM747837); *S. otites, Ochoa 11779*, CIP 761276, Venezuela, Táchira (HM747402, HM747403; HM747540, HM747541, HM747542; HM747669, HM747670, HM747671; HM747780, HM747781; HM747890). Map Locality 12: *S. garcia-barrigae, López et al. CCC 5170,* PI 498158, Colombia, Norte de Santander (HM747359, HM747360, HM747361; HM747502, HM747503; HM747628, HM747629; HM747754, HM747755; HM747856, HM747857); *S. orocense, Spooner et al. 1304,* PI 583307, Colombia, Norte de Santander (HM747398, HM747399, HM747400, HM747401; HM747537, HM747538, HM747539; HM747666, HM747667, HM747668; HM747779; HM747888, HM747889). Map Locality 13: *S. flahaultii, Lopez et al. CCC 5255,* PI 583316, Colombia, Boyacá (HM747352, HM747353; HM747495, HM747496; HM747621, HM747622; HM747746, HM747747, HM747748; HM747852). Map Locality 15: *S. flahaultii, Lopez et al. CCC 5272,* PI 597674, Colombia, Boyacá (HM747357, HM747358; HM747500, HM747501; HM747626, HM747627; HM747752, HM747753; HM747854, HM747855). Map Locality 16: *S. flahaultii, Castillo et al. 1272,* PI 570620, Colombia, Cundinamarca (HM747354, HM747355, HM747356; HM747497, HM747498, HM747499; HM747623, HM747624, HM747625; HM747749, HM747750, HM747751; HM747853). Map Locality 18: *S. colombianum, Castillo et al. 1212,* PI 567831, Colombia, Caldas (HM747350, HM747351; HM747493, HM747494; HM747618, HM747619, HM747620; HM747743, HM747744, HM747745; HM747850, HM747851); *S. lobbianum, Castillo et al. 1211,* PI 567840, Colombia, Caldas (HM747362, HM747363, HM747364; HM747504, HM747505; HM747630, HM747631; HM747756, HM747757, HM747758; HM747858, HM747859). Map Locality 19: *S. colombianum, Castillo et al. 1202,* PI 587827, Colombia, Quindío (HM747348, HM747349; HM747491, HM747492; HM747616, HM747617; HM747741, HM747742; HM747848, HM747849). Map Locality 20: *S. colombianum, Lopez et al. CCC 5218,* PI 583312, Colombia, Valle (HM747322, HM747323, HM747324; HM747466, HM747467, HM747468; HM747595, HM747596; HM747729; HM747830, HM747831). Map Locality 21: *S. colombianum, Lopez et al. CCC 5284,* PI 583319, Colombia, Cauca (HM747325, HM747326, HM747327, HM747328; HM747469, HM747470, HM747471; HM747597, HM747598, HM747599; HM747730; HM747832, HM747833). Map Locality 23: *S. moscopanum, Castillo et al. 1243,* PI 567843, Colombia, Cauca (HM747390, HM747391, HM747392, HM747393, HM747394; HM747531, HM747532, HM747533, HM747534; HM747659, HM747660, HM747661, HM747662; HM747776; HM747883, HM747884, HM747885, HM747886). Map Locality 24: *S. colombianum, López 10,* PI 583322, Colombia, Cauca (HM747329, HM747330; HM747472, HM747473, HM747474, HM747475; HM747600, HM747601, HM747602, HM747603; HM747731; HM747834, HM747835, HM747836); *S. sucubunense, Castillo et al. 1255,* PI 583320, Colombia, Cauca (HM747419, HM747420, HM747421, HM747422; HM747554, HM747555, HM747556; HM747684, HM747685, HM747686, HM747687; HM747798; HM747901, HM747902, HM747903, HM747904). Map Locality 25: *S. colombianum, Hawkes 2544,* PI 320346, Colombia, Nariño (HM747334, HM747335, HM747336, HM747337; HM747478, HM747479, HM747480; HM747606, HM747607; HM747735, HM747736; HM747838, HM747839). Map Locality 26: *S. colombianum, López et al. CCC 5143,* PI 498151, Colombia, Nariño (HM747320, HM747321; HM747464, HM747465; HM747592, HM747593, HM747594; HM747728; HM747828, HM747829). Map Locality 27: *S. colombianum, Spooner et al. 5025,* PI 561633, Ecuador, Pichincha (HM747338, HM747339; HM747481, HM747482; HM747608, HM747609; HM747737, HM747738; HM747840, HM747841). Map Locality 28: *S. moscopanum, Spooner et al. 5005,* PI 561626, Ecuador, Pichincha (HM747381, HM747382, HM747383; HM747520, HM747521, HM747522; HM747647, HM747648, HM747649, HM747650; HM747773; HM747873, HM747874, HM747875, HM747876). Map Locality 29: *S. colombianum, Spooner et al. 5119,* PI 561647, Ecuador, Napo (HM747344, HM747345, HM747346, HM747347; HM747487, HM747488, HM747489, HM747490; HM747612, HM747613, HM747614, HM747615; HM747740; HM747845, HM747846, HM747847). Map Locality 31: *S. tundalomense, Ochoa et al. 11004,* PI 473474, Ecuador, Azuay (HM747423, HM747424, HM747425, HM747426; HM747557, HM747558, HM747559; HM747688,

HM747689, HM747690, HM747691; HM747799; HM747905, HM747906, HM747907, HM747908); *S. tundalomense, Ochoa 13359*, CIP 761592, Ecuador, Azuay (HM747427, HM747428, HM747429, HM747430; HM747560, HM747561, HM747562, HM747563; HM747692, HM747693, HM747694, HM747695; HM747800; HM747909, HM747910, HM747911). Map Locality 32: *S. colombianum, Spooner et al. 5062,* PI 561640, Ecuador, Cañar (HM747340, HM747341, HM747342, HM747343; HM747483, HM747484, HM747485, HM747486; HM747610, HM747611; HM747739; HM747842, HM747843, HM747844); *S. moscopanum, Spooner et al. 5139,* PI 561659, Ecuador, Cañar (HM747384, HM747385, HM747386; HM747523, HM747524, HM747525, HM747526, HM747527; HM747651, HM747652, HM747653, HM747654; HM747774; HM747877, HM747878, HM747879). Map Locality 33: *S. moscopanum, Spooner et al. 5040,* PI 567812, Ecuador, Loja (HM747387, HM747388, HM747389; HM747528, HM747529, HM747530; HM747655, HM747656, HM747657, HM747658; HM747775; HM747880, HM747881, HM747882). Map Locality 34: *S. trinitense, Ochoa et al. 16252*, CIP 763642, Peru, Cajamarca (HM072723; HM073151; HM072947; HM073366; HM073593, HM073592). Map Locality 35: *S. nubicola, Ochoa 13335*, CIP 761585, Peru, La Libertad (HM747395, HM747396, HM747397; HM747535, HM747536; HM747663, HM747664, HM747665; HM747777, HM747778; HM747887). Map Locality 37: *S. laxissimum, Ochoa et al. 11855*, PI 498252, Peru, Junín (HMO72646; HM073092; HM072882; HM073303; HM073524). Map Locality 38: *S. buesii, Spooner et al. 7235*, CIP 762855, Peru, Cuzco (HM747319; HM747463; HM747591; HM747727; HM747827); *S. santolallae, Spooner et al. 7228*, CIP 762848, Peru, Cuzco (HM747417; HM747552; HM747682; HM747795, HM747796; HM747899); *S. urubambae, Spooner et al. 7217*, CIP 762836 (HM747441; HM747573; HM747706; HM747807; HM747922). Map Locality 39: *S. santolallae, Spooner et al. 7237*, CIP 762857, Peru, Cuzco (HM747418; HM747553; HM747683; HM747797; HM747900). Map Locality 40: *S. pillahuatense, Spooner et al. 7220*, CIP 762840, Peru, Cuzco (HM747412, HM747413; HM747549; HM747678; HM747791; HM747896); *S. santolallae, Hawkes et al. 5103*, CIP 760350, Peru, Cuzco (HM747415, HM747416; HM747551; HM747680, HM747681; HM747793, HM747794; HM747898); *S. urubambae, Spooner et al. 7225*, CIP 762845, Peru, Cuzco (HM747442; HM747574; HM747707; HM747808; HM747923). Map Locality 41: *S. buesii, Ochoa 13637*, CIP 761690, Peru, Cuzco (HM747318; HM747462; HM747590; HM747726; HM747826); *S. urubambae, Ochoa 13778*, CIP 761795, Peru, Cuzco (HM747439; HM747571; HM747703; HM747805; HM747919, HM747920); *S. urubambae, Ochoa 13778A*, CIP 761796, Peru, Cuzco (HM072732; HM073160; HM072956; HM073373; HM073603); *S. urubambae, Ochoa 13779*, CIP 761797, Peru, Cuzco (HM747318; HM747462; HM747590; HM747726; HM747826); *S. urubambae, Ochoa 13778A1*, CIP 763314, Peru, Cuzco (HM747436; HM747568; HM747700; HM747802; HM747915, HM747916); *S. urubambae, Ochoa 13778A2*, CIP 763315, Peru, Cuzco (HM747437; HM747569; HM747701; HM747803; HM747917). Map Locality 42: *S. limbaniense, Ochoa 12594,* CIP 752552, Peru, Puno (HM747374, HM747375; HM747515; HM747642; HM747768; HM747868); *S. limbaniense, Ochoa 14288*, CIP 761967, Peru, Puno (HM747376, HM747377; HM747516; HM747643; HM747769; HM747869); *S. limbaniense, Ochoa 14290*, CIP 761968, Peru, Puno (HM747378; HM747517; HM747644; HM747770; HM747870); *S. limbaniense, Ochoa 14292*, CIP 761970, Peru, Puno (HM747379; HM747518; HM747645; HM747771; HM747871); *S. limbaniense, Ochoa 15601*, CIP 762336, Peru, Puno (HM747371; HM747513; HM747639, HM747640; HM747766; HM747866); *S. limbaniense, Spooner et al. 7205,* CIP 762824, Peru, Puno (HM072644; HM073090, HM072879; HM072880; HM073301; HM073521). Map Locality 43: *S. violaceimarmoratum, Ochoa 11901*, CIP 761342, Bolivia, La Paz (HM747449; HM747580; HM747713; HM747815; HM747930). Map Locality 44: *S. violaceimarmoratum, Gandarillas s. n.*, CIP 763067, Bolivia, La Paz (HM747443; HM747575; HM747708; HM747809; HM747924, HM747925); *S. violaceimarmoratum, Hawkes et al. 5040*, CIP 760330, Bolivia, La Paz (HM747447; HM747578; HM747711; HM747812; HM747928); *S. violaceimarmoratum, Hawkes et al. 5042*, CIP 760331, Bolivia, La Paz (HM747448; HM747579; HM747712; HM747813, HM747814; HM747929); *S. violaceimarmoratum, Spooner et al. 6731,* PI 631229, Bolivia, La Paz (HM747444, HM747445; HM747576; HM747709; HM747810; HM747926); *S. violaceimarmoratum, Van Soest et al. 7*, CIP 760563, Bolivia, La Paz (HM747451, HM747452; HM747582; HM747715, HM747716; HM747817; HM747932, HM747933). Map Locality 45: *S. violaceimarmoratum, Hawkes et al. 4436*, CIP 760240, Bolivia, Cochabamba (HM747446; HM747577; HM747710; HM747811; HM747927); *S. violaceimarmoratum, Hawkes et al. 4474,* PI 473396, Bolivia, Cochabamba (HM747450; HM747581; HM747714; HM747816; HM747931). Not mapped: *S. laxissimum, Erwin Baur Sortiment 1888,* PI 283088, Peru, Cuzco (HM747380; HM747519; HM747646; HM747772; HM747872); *S. limbaniense, Ochoa 5123,* PI 473468, Peru (HM747372, HM747373; HM747514; HM747641; HM747767; HM747867); *S. santolallae, CPC2078.2,* PI 195168, Peru, Cuzco (HM747414; HM747550; HM747679; HM747792; HM747897); *S. tundalomense, Ochoa 13396*, CIP 761606 (HM747431, HM747432, HM747433, HM747434, HM747435; HM747564, HM747565, HM747566, HM747567; HM747696, HM747697, HM747698, HM747699; HM747801; HM747912, HM747913, HM747914); *S. urubambae, Ochoa 13614,* CIP 761676 (HM747438; HM747570; HM747702; HM747804; HM747918); *S. violaceimarmoratum, Zavaleta 1579*, PI 498314, (HM072734; HM073162; HM072958; HM073375; HM073605).