Phylogeny of *Solanum* series *Piurana* and related species in *Solanum* section *Petota* based on five conserved ortholog sequences

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Abstract Taxonomic complexity in wild potatoes (*Solanum* L. sect. *Petota* Dumort.) has led to widely conflicting treatments. *Solanum* ser. *Piurana* Hawkes is one of 21 series recognized in *S.* sect. *Petota* in the latest comprehensive taxonomic treatment by Hawkes in 1990. They are distributed from southern Colombia south through Ecuador to central Peru. The taxonomic limits of the series and validity of its constituent species were not resolved with previous studies. In the present study, a set of five conserved orthologous DNA markers (cos II) with 5342 bp of aligned length were used to infer the phylogeny of putative members of *Solanum* ser. *Piurana* and outgroups. The results agreed with a three-clade topology shown by previous studies within *S.* sect. *Petota*. *Solanum* ser. *Piurana* is expanded to include some species formerly included in *S.* sers. *Conicibaccata*, *Cuneoalata*, *Ingaefolia*, *Olmosiana*, *Simplicissima*, *Tuberosa* and *Yungasensa*. This expanded group is supported morphologically by the presence of moniliform tubers and coriaceous leaves in most species.

Keywords COSII markers; phylogeny; Solanaceae; *Solanum*; wild potatoes

Supplementary material The Appendix is available in the free Electronic Supplement to the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

■ INTRODUCTION

Wild potatoes (*Solanum* sect. *Petota* Dumort.; from now on referred to as "sect. *Petota*") are morphologically diverse, growing in a variety of habitats from the southwestern United States to central Argentina and Chile. The group is taxonomically complex, and there are widely conflicting taxonomic treatments regarding both the number of species recognized and their affiliation to series (Spooner, 2009). Hawkes (1990) recognized 232 species distributed in 21 series but the most recent estimate is 110 species divided into three main clades (Spooner & al., 2009).

About 70% of the wild potato species are diploid ($2n = 2x = 24$) with the remaining species polyploid at the triploid, tetraploid, pentaploid, and up to the hexaploid ($2n = 6x = 72$) level (Hijmans & al., 2007). Wild potatoes have proven to be a source of important traits that can be utilized in breeding programs of cultivated potatoes, and several species have been used as sources of disease resistance, stress tolerance and improved tuber quality in the cultivated potato (e.g., Ortiz, 2001; Bradshaw & al., 2006; Jansky, 2009). According to Hawkes (1990), the morphological gaps among wild potato species can be explained by several isolating factors including ecogeographical isolation, flowering times, varying ploidy levels, endosperm balance number (EBN, a system of sexual compatibility based on ratios of maternal/paternal genomes in the endosperm), and genome differences.

Although *Solanum* ser. *Piurana* (from now on referred to as "ser. *Piurana*") traditionally has contained some of the morphologically most distinctive species in the section, the definition and limits of this series has always been controversial (Ames & al., 2008). The features of ser. *Piurana* used by all major taxonomists of sect. *Petota* (Correll, 1962; Hawkes, 1990; Ochoa, 1999) are globose to ovoid fruits and coriaceous glossy leaves. However, these traits vary in expression across the different series so that is difficult to decide what species to include in ser. *Piurana.* All the above authors have expressed doubts about the limits of ser. *Piurana*. For example, Correll (1962: 139) stated: "This series [*Piurana*], probably more than any of the others, may be considered a catchall. Paradoxically, its component species are held together not so much by their similarity as by their differences." Correll (1962) recognized 15 species in the series, Hawkes (1990) 15, and Ochoa (1999) 13; however, they recognized only six species in common within the series: *S. acroglossum* Juz., *S. cantense* Ochoa, *S. cyanophyllum* Corr. (= *S. andreanum* Baker), *S. hypacrarthrum* Bitter, *S. piurae* Bitter and *S. solisii* Hawkes. Ames & al. (2008) showed that the only distinctive features of ser. *Piurana* might be the coriaceous and glossy leaves (Correll, 1962; Hawkes, 1990; Ochoa, 1999) and moniliform tubers (arranged like beads on a string), in contrast to the usual placement of single tubers at the end of a stolon.

In sect. *Petota*, studies based on plastid DNA restriction sites have been conducted for the North and Central American species (Spooner & Sytsma, 1992) and the South American species (Spooner & Castillo, 1997). These studies defined four clades in sect. *Petota*: (1) the North and Central American diploid species, exclusive of *S. bulbocastanum* Dun., *S cardiophyllum* Lindl. and

S. verrucosum Schltdl.; (2) *S. bulbocastanum* and *S. cardiophyllum*; (3) all examined members of the South American ser. *Piurana* and some South American species placed in other series; (4) all remaining South American species, *S. verrucosum*, and the North and Central American polyploid species. Castillo & Spooner (1997) also examined phylogenetic relationships using plastid DNA restriction site variation and plant morphology. The results showed that some of the species traditionally placed in *Solanum* ser. *Conicibaccata* (*S. chomatophilum* Bitter, *S. contumazaense* Ochoa, *S. paucijugum* Bitter, and *S. tuquerrense* Hawkes) were members of ser. *Piurana*.

Additional phylogenetic studies based on nuclear gene sequences of the granule-bound starch synthase gene (GBSSI or *waxy*) (Spooner & al., 2008) nitrate reductase (Rodríguez & Spooner, 2009), and COSII genes (Rodríguez & al., 2009) identified three clades in sect. *Petota*, with clades 1 and 2 of the plastid data collapsing into a single clade (now called clade 1+2) and clades 3 and 4 in agreement with the plastid phylogenies mentioned above. The COSII study (Rodríguez & Spooner, 2009) was particularly useful because (1) it produced a fully resolved (99%/100% bootstrap) phylogeny using representatives of all clades in sect. *Petota*, and (2) it identified reduced subsets of COSII markers to use on expanded sets of taxa within each clade in sect. *Petota*. All of these independent studies suggested that ser. *Piurana* had a monophyletic origin, but the reduced number of species included in those studies limited comprehensive conclusions about the number of species included within it.

Single- to low-copy nuclear DNA markers are currently used for phylogenetic reconstruction. In order to examine the species boundaries and relationships of all members of the expanded concept of ser. *Piurana* we used a specific subset of markers called conserved ortholog set II (COSII) markers. These markers were developed from throughout the nuclear genome, and specific primers were designed to amplify either intronic or exonic regions of orthologues in the Solanaceae (Fulton & al., 2002; Wu & al., 2006). In the present study we used five of these COSII markers identified by Rodriguez & al. (2009) as the most informative for phylogenetic studies in sect. *Petota*.

■ MATERIALS AND METHODS

Taxon sampling, DNA isolation, generation and alignment of DNA sequences. — This study examined 40 wild potato species (173 accessions), 37 of them diploid and three polyploid (Table 1) as putative members of ser. *Piurana* based on previous hypothesis from molecular and morphological data (Ames & al., 2008). Eighteen additional species (22 accessions) were included in the analysis to represent other clades of *Solanum* sect. *Petota* (Spooner & Castillo, 1997). One accession of

Table 1. List of species included in the analysis indicating the number of accessions included in the analysis, their ploidy level, endosperm balance number (EBN), their series classification by Hawkes (1990) and their major geographic area of distribution. Accession-specific information including the collector number, location, and genebank number are in the supplementary Appendix.

Species	Number of accessions evaluated	Ploidy	EBN	Series assignation (Hawkes, 1990)	Major geographical area distribution
Potential ingroup species					
Solanum acroglossum Juz.	1	2*x*	2	*Piurana*	Peru
S. acroscopicum Ochoa	1	2*x*	Unk.	*Tuberosa*	Peru
S. albornozii Correll	4	2*x*	2	*Piurana*	Ecuador
S. anamatophilum Ochoa	1	2*x*	2	*Cuneoalata*	Peru
S. andreanum Baker	10	2*x*	2	*Tuberosa*	Colombia, Ecuador
S. augustii Ochoa	2	2*x*	1	*Tuberosa*	Peru
S. cajamarquense Ochoa	8	2*x*	1	*Tuberosa*	Peru
S. cantense Ochoa	5	2*x*	2	*Piurana*	Peru
S. chancayense Ochoa	2	2*x*	1	*Tuberosa*	Peru
S. chilliasense Ochoa	2	2*x*	2	*Piurana*	Ecuador
S. chiquidenum Ochoa	10	2*x*	2	*Tuberosa*	Peru
S. chomatophilum Bitter	27	2*x*	2	*Conicibaccata*	Colombia, Ecuador, Peru
S. contumazaense Ochoa	1	2*x*	2	*Conicibaccata*	Peru
S. dolichocremastrum Bitter	12	2*x*	1	*Tuberosa*	Peru
S. guzmanguense Whalen & Sagást.	2	2*x*	1	*Tuberosa*	Peru
S. huancabambense Ochoa	6	2*x*	2	*Yungasensa*	Peru
S. huarochiriense Ochoa	4	2*x*	2	*Tuberosa*	Peru
S. humectophilum Ochoa	1	2*x*	1	*Tuberosa*	Peru
S. hypacrarthrum Bitter	5	2*x*	1	*Piurana*	Peru

Table 1. Continued.

Species	Number of accessions evaluated	Ploidy	EBN	Series assignation (Hawkes, 1990)	Major geographical area distribution
S. immite Dunal	3	2*x*	1	*Tuberosa*	Peru
S. infundibuliforme Phil.	2	2*x*	2	*Cuneoalata*	Argentina, Bolivia
S. ingifolium Ochoa	1	2*x*	1	*Ingifolia*	Peru
S. jalcae Ochoa	7	2*x*	2	*Piurana*	Peru
S. marinasense Vargas	11	2*x*	2	*Tuberosa*	Peru
S. minutifoliolum Correll	1	2*x*	1	*Tuberosa*	Ecuador
S. mochiquense Ochoa	8	2*x*	1	*Tuberosa*	Peru
S. multiinterruptum Bitter	3	2*x*	2	*Tuberosa*	Peru
S. olmosense Ochoa	1	2*x*	2	*Olmosiana*	Ecuador
S. pascoense Ochoa	1	2*x*	2	*Piurana*	Peru
S. paucijugum Bitter	3	4*x*	2	*Conicibaccata*	Ecuador
S. paucissectum Ochoa	6	2*x*	2	*Piurana*	Peru
S. peloquinianum Ochoa	3	2*x*	2	*Cuneoalata*	Peru
S. piurae Bitter	3	2*x*	2	*Piurana*	Peru
S. scabrifolium Ochoa	1	2*x*	Unk.	*Tuberosa*	Peru
S. simplicissimum Ochoa	2	2*x*	1	—	Peru
S. sogarandinum Ochoa	5	2*x*	2	*Megistacroloba*	Peru
S. solisii Hawkes	1	4*x*	Unk.	*Piurana*	Ecuador
S. trinitense Ochoa	1	2*x*	1	*Conicibaccata*	Peru
S. tuquerrense Hawkes	3	4*x*	2	*Piurana*	Colombia, Ecuador
S. ×*blanco-galdosii* Ochoa	3	2*x*	2	*Piurana*	Peru
Outgroup species representative of clades 1+2					
S. bulbocastanum Dunal	1	2*x*	1	*Bulbocastana*	Guatemala, Mexico
S. stenophyllidium Bitter	2	2*x*	1	*Pinnatisecta*	Mexico
S. trifidum Correll	1	2*x*	1	*Pinnatisecta*	Mexico
S. tarnii Hawkes & Hjert.	3	2*x*	Unk.	*Pinnatisecta*	Mexico
S. ×*sambucinum* Rydb.	1	2*x*	Unk.	*Pinnatisecta*	Mexico
Outgroup species representative of clade 4					
S. ambosinum Ochoa	1	2*x*	2	*Tuberosa*	Peru
S. brevicaule Bitter	1	2*x*	2	*Tuberosa*	Bolivia
S. buesii Vargas	1	2*x*	2	*Conicibaccata*	Peru
S. bukasovii Juz.	1	2*x*	2	*Tuberosa*	Peru
S. kurtzianum Bitter & Wittm.	1	2*x*	2	*Tuberosa*	Argentina
S. laxissimum Bitter	1	2*x*	2	*Conicibaccata*	Peru
S. leptophyes Bitter	1	2*x*, 4*x*	2, 4	*Tuberosa*	Argentina, Bolivia, Peru
S. limbaniense Ochoa	1	2*x*	2	*Conicibaccata*	Peru
S. raphanifolium Cárdenas & Hawkes	1	2*x*	2	*Megistacroloba*	Peru
S. urubambae Juz.	1	2*x*	2	*Conicibaccata*	Peru
S. verrucosum Schltdl.	2	2*x*	2	*Tuberosa*	Mexico
S. violaceimarmoratum Bitter	1	2*x*	2	*Conicibaccata*	Bolivia
S. yungasense Hawkes	1	2*x*	2	*Yungasensa*	Bolivia
Far outgroup species					
S. etuberosum Lindl.	1	2*x*	1	*Etuberosa*	Chile
S. fernandezianum Phil.	3	2*x*	1	*Etuberosa*	Argentina

S. etuberosum and three of *S. fernandezianum* were included as outgroups based on previous studies using plastid DNA (Spooner & al., 1993) and nuclear DNA (Rodríguez & al., 2009). In total, 199 different accessions were analyzed; including allelic variants the dataset included 297 OTUs. Five pairs of COSII primers were chosen based on results of Rodríguez & al. (2009). All the PCR products for the polyploidy species were cloned and ten clones were sequenced for each accession. For the diploids only PCR products that showed a second faint band or unreadable sequences were cloned and five clones were sequenced for each accession. The primer sequences, the position on the potato chromosomes, and the PCR amplification parameters, DNA extraction, PCR, cloning, sequencing and alignment are described in Rodríguez & al. (2009).

Statistical tests. — The measure of phylogenetic signal for each dataset was estimated by the g1 statistic in PAUP* using 10,000 random trees. The significance of the statistic was assessed following Hillis & Huelsenbeck (1992). Incongruence among the five COSII regions were assessed using the incongruence length difference (ILD) test (Farris & al., 1994) implemented as the homogeneity test in PAUP*. The test was performed with 1000 replicate partitions; each partition was subjected to heuristic parsimony searches with 100 random taxon addition replicates using TBR branch swapping, keeping no more than 1 tree per random replicate. Templeton tests (Templeton, 1983) as implemented in PAUP* were used to explore alternative topologies in a maximum parsimony framework.

Phylogenetic analyses. — Phylogenetic analyses based on maximum parsimony were conducted using PAUP version 4.0b10 (Swofford, 2002). The most parsimonious trees were found by heuristic searches under Fitch criteria and equally weighted characters. A heuristic search was conducted using TBR branch swapping on 10,000 random taxon addition sequences. A rooted strict-consensus tree was obtained using *S. etuberosum* and *S. fernandezianum* as outgroups designating them a monophyletic sister group to the ingroup of the remaining species in sect. *Petota*. To estimate clade support, 1000 bootstrap replicates were subjected to heuristic searches using TBR branch swapping on 1000 random taxon addition sequences.

To select the most appropriate model of evolution for maximum likelihood analysis, a total of 56 models were tested through Modeltest (Posada & Crandall, 1998). The maximum likelihood phylogeny was estimated using RaxML v.7.0.4 (Randomized Axelerated Maximum Likelihood; Stamatakis, 2006) web-based on the CIPRES v.1.13 cluster at the San Diego Supercomputer Center. The rapid RAxML bootstrapping algorithm was used to obtain bootstrap support values in a maximum likelihood framework performing 2000 replicates. The concatenated dataset was uploaded into the CIPRES portal and the five COSII regions were analyzed under the GTR model that is fixed in RaxML but individually optimized for each region. A rooted tree was generated using *S. etuberosum* and *S. fernandezianum* as outgroups.

Bayesian phylogenetic analysis was conducted using MrBayes online version at CIPRES Portal v.1.13. As the analysis on CIPRES does not allow defining partitions, the HKY+∂ model was used for the concatenated dataset; this model was found to be the most appropriate model for DNA substitutions in all five partitions. Tree searching was performed running four linked chains, initiated from random trees with a sequential heat of 0.05 (determined empirically) for four million generations with trees sampled every 1000 generations. One quarter of the initially generated trees were discarded manually as it is not possible to automatically define the burn-in fraction. The search for trees was repeated four times and the majority rule consensus trees from each run were visually compared and then mixed to generate a majority rule consensus tree that was taken as the best representation of the posterior distributions of tree topology and model parameters (Huelsenbeck & Ronquist, 2001).

Trait reconstruction. — The circumscription of series *Piurana*, and later hypotheses of an expanded series *Piurana* (Correll, 1962; Hawkes, 1990; Ochoa, 1999; Ames & al., 2008), was partly based on two morphological characters, moniliform tubers and a coriaceous leaf texture (Ames & al., 2008). Leaf texture is a consistent character within accessions of different species, so there were no issues during the scoring of the character. However, moniliform and terminal tubers sometimes are present even in different genotypes of the same accession, and some accessions of *S. chomatophilum* did not form tubers in our greenhouse conditions (Ames & al., 2008). In order to score the character, only the most frequent character for tubers within a species was reconstructed in the phylogeny. Only one or two accessions of each species were considered for the phylogeny for both leaves and tubers. To better visualize patterns of evolution these two traits were treated as discrete characters and traced on a phylogeny using McClade v.4.0 (Maddison & Maddison, 2001). The phylogeny was generated through maximum likelihood and included only one representative accession for each species (except for *S. chiquidenum* and *S. stenophyllidium* which included two accessions). Character states for tubers were: (0) no tuber formation, (1) moniliform tubers, (2) tubers placed at the end of the stolons. The character states for leaves were: (0) chartaceous and (1) coriaceous. Characters were treated as unordered and gains and losses of characters were equally weighted.

■ RESULTS

Sequence alignment and allelic variants. — The aligned DNA sequence was 5342 bp long (see Table 2 for details). The sequence length ranged from 641 to 694 bp for COSII 1C, 448 to 459 bp for COSII 3, 700 to 1198 bp for COSII 3C, 383 to 2264 bp for COSII 9 and 483 to 682 bp for COSII 11. Of the 193 diploid accessions, 109 showed a single sequence type and 84 showed two sequence types. Of the six tetraploid accessions, one showed only two sequence types, two showed three types, and three showed four types. No stop codons were found after translating exons. The aligned matrix is available in TreeBase (www.treebase.org) or on request from the authors.

Statistical tests and model selection. — The number of characters for each of the five COSII regions, the number of variable, parsimony informative characters, and the measure of the phylogenetic signal and the number and length of trees is described in Table 2.

Table 2. Statistics for individual and combined datasets including the 297 OTUs.

Region	No. of total characters	No. of variable characters	No. of parsimony informative characters	Consistency index/retention index	g1	Tree length	No. of most parsimonious trees
COSII 1C	720	327	236	0.61/0.93	–0.15*	663	1207
COSII 3	462	90	69	0.86/0.99	–0.46*	118	9524
COSII 3C	1198	303	212	0.62/0.91	–0.18*	583	788
COSII 9	2264	637	424	0.77/0.95	–0.11*	949	499
COSII 11	698	269	190	0.62/0.95	–0.20*	522	2236
5 COSII concat.	5342	1626	1131	0.41/0.82	–0.16*	4542	2

* significant phylogenetic signal ($P < 0.01$).

The ILD test revealed that there was a significant level of incongruence among the five datasets (COSII1C-COSII3, $P = 0.001$; COSII1C-COSII3C, $P = 0.001$; COSII1C-COSII9, $P = 0.001$; COSII1C-COSII11, $P = 0.001$; COSII3-COSII3C, $P = 0.001$; COSII3-COSII9, $P = 0.001$; COSII3-COSII11, $P = 0.001$; COSII3C-COSII9, $P = 0.001$; COSII3C-COSII11, $P = 0.001$; COSII9-COSII11, $P = 0.001$). However, combining the five COSII regions resulted in a better-resolved tree with higher branch support for clades (Fig. 1 on fold-out following page 1096) than either of the single COSII partitions.

The model test analysis produced the same model (HKY + G) for the five partitions. This model was used for the Bayesian phylogenetic analysis on both CIPRES and MrBayes. CIPRES interface does not allow setting the model of nucleotide substitution when performing maximum likelihood analysis.

Phylogenetic analyses. — Maximum likelihood analysis of the concatenated dataset produced a single tree of score –lnL= –38987.026 (Fig. 1). Maximum parsimony analysis produced two equally most-parsimonious trees (length = 4542, CI = 0.41, RI = 0.82). A strict consensus tree was generated (tree not shown) and bootstrap values calculated in a maximum parsimony framework are shown in Fig. 1.

The results obtained through maximum likelihood identified three main clades. These three clades were designated as 1+2, 3 and 4, consistent with the four-clade plastid designation of Spooner & Castillo (1997), with clade 1+2 containing the North and Central American diploid wild potatoes sister to clade 3 (sect. *Piurana* members) and clade 4 (close relatives of the cultivated potato). The maximum likelihood support values for clades 3 (52%) and 4 (58%) were low. Bayesian analysis (not shown) placed clades 3 and 4 as a polytomy, resolving only clade 1+2. The results obtained through maximum parsimony analysis did not show clades 3 and 4 as sister clades, but resolved clade 4 as being part of clade 3 (not shown).

Clade 1+2 had identical topologies in all three analyses. The topology within clade 4 was nearly identical between maximum likelihood and maximum parsimony; this clade included all the species that were identified as members of clade 4 in previous studies (Spooner & Castillo, 1997; Rodríguez & Spooner, 2009; Spooner & al., 2008), as well as *S. marinasense* and *S. sogarandinum*. *Solanum sogarandinum* was considered a putative member of ser. *Piurana* based on plastid DNA restriction site data (Spooner & Castillo, 1997), and is the only species in these studies showing discordance in its phylogenetic placement within or outside ser. *Piurana* between the plastid and nuclear DNA datasets. *Solanum marinasense* was considered a putative member of ser. *Piurana* based solely on morphological data due to the variability among accessions regarding glossy leaves or/and moniliform tubers (Ames & al., 2008). Clade 3 of the present analysis therefore, contains the majority of the species considered to be putative members of ser. *Piurana* based on hypotheses of Ames & al. (2008).

The internal topology of clade 3 showed a well-supported subclade (84% bootstrap) containing species distributed in Colombia (*S. andreanum*) and Ecuador (*S. andreanum*, *S. albornozii*, *S. olmosense*, *S. minutifoliolum*, *S. solisii*), as well as alleles from the two polyploid species *S. paucijugum* (distributed throughout Ecuador) and *S. tuquerrense* (distributed from Ecuador to southern Colombia) (Fig. 1). The only species from this geographical area not included in this clade is the Ecuadorian *S. chilliasense*; in addition, there are Ecuadorian localities of *S. chomatophilum* but they were not available for sampling. The remaining 30 species of clade 3 are distributed from central to northern Peru and no other well supported clades with geographical associations were found.

Diploid species. — Of the 34 diploid species grouped within clade 3, eleven were represented by only one accession (many of the species in sect. *Petota* are rare in nature and in genebanks), and 23 were represented by two or more accessions (Table 1). From the eleven diploid species represented by only one accession, five showed only one sequence (*S. contumazaense*, *S. humectophilum*, *S. ingifolium*, *S. minutifoliolum*, *S. olmosense*). Three species (*S. acroglossum*, *S. acroscopicum*, *S. scabrifolium*) showed two sequences (alleles) that grouped together. Three species showed two alleles that fell in clades of different species. For *S. anamatophilum* one allele fell in a clade with *S. multiinterruptum* and the other with *S. peloquinianum*; for *S. pascoense* one allele fell in a clade with *S. chomatophilum* and *S. jalcae* and the other in a clade comprised by some accessions of *S. jalcae* and *S. chiquidenum*; and for *S. trinitense* one allele was found in a clade with *S. multiinterruptum* and the other one with *S. augustii* (Fig. 1). The Templeton test rejected the possibility of both alleles of *S. anamatophilum* grouping together ($P = 0.0002–0.0005$)

and it also rejected the possibility of both alleles from *S. pascoense* grouping together (P = 0.0026–0.0056); however, the possibility of both alleles from *S. trinitense* grouping together was not rejected (P = 0.5221–0.6085). All the Templeton tests performed were based on constraints applied to the ML tree topology (Fig. 1)

Fourteen diploid species out of the 23 species represented by two or more accessions showed all of their accessions and alleles forming monophyletic groups: *S. albornozii*, *S. augustii*, *S. cajamarquense*, *S. cantense*, *S. chancayense*, *S. chilliasense*, *S. dolichocremastrum*, *S. guzmanguense*, *S. huancabambense*, *S. huarochiriense*, *S. hypacrarthrum*, *S. immite*, *S. multiinterruptum*, and *S. simplicissimum*. The clades showed high support values except for *S. multiinterruptum* (Fig. 1). There was one accession with two alleles of *S. sogarandinum* within the clade of *S. dolichocremastrum*. The Templeton test rejected forcing this accession of *S. sogarandinum* into a clade with the remaining accessions of this species in clade 4 ($P < 0.0001$).

The alleles for the nine remaining diploid species represented by more than two accessions did not group together. For *S. andreanum*, eight accessions out of the ten included in the analysis formed a clade with high support. One accession clustered with alleles of the polyploid species *S. tuquerrense* in the Ecuador-Colombia clade. One allele of one accession fell with alleles of *S. solisii* and *S. paucijugum* in the Ecuador-Colombia clade and the other within a poorly resolved clade of several species mainly distributed in northern Peru that also contained the remaining alleles of the polyploid species *S. paucijugum* and *S. tuquerrense*. The Templeton test rejected the hypothesis of all the accessions and alleles of *S. andreanum* forming a clade ($P < 0.0001$). For *S. chiquidenum*, five of the ten accessions fell in the same clade with *S. acroglossum*, *S. cantense*, *S. huarochiriense*, *S. paucissectum* and with some accessions of *S. chomatophilum* and *S. jalcae*. The other five fell in a different clade, with *S. ×blanco-galdosii*, S. *cajamarquense*, *S. humectophilum* and some accessions of *S. jalcae*. The Templeton test rejected the possibility of all the accessions of *S. chiquidenum* forming a clade (P = 0.0104–0.0184).

Only four of the 27 accessions of *S. chomatophilum* formed a clade with high support; the remaining accessions grouped in several small clades with low support and sometimes they included accessions of *S. jalcae*. The hypothesis of clustering all of the accessions from *S. chomatophilum* together was not supported by the Templeton test ($P < 0.0001$) and similarly rejected all the accessions of *S. jalcae* grouping together ($P < 0.0001$).

Of the six accessions of *S. paucissectum* only three (with one allele each) plus one allele from a fourth accession grouped together; the other allele grouped with *S. chomatophilum*. A fifth accession grouped with alleles from *S. paucijugum* and the sixth accession grouped with *S. piurae* and *S. chilliasense*. The Templeton test rejected the possibility of all accessions of *S. paucissectum* grouping together ($P < 0.0001$).

The three accessions of *S. piurae* did not form a clade; instead the three of them were part of a clade with *S. chilliasense* and one accession of *S. paucissectum* as previously mentioned. The Templeton test rejected the hypothesis of *S. piurae* as monophyletic ($P < 0.0001$).

The three accessions of *S. peloquinianum* fell in different well supported clades: two of them plus one allele of the third in a clade that also included one allele of *S. anamatophilum*, and the other allele from the third accession with two out of the three accessions of *S. ×blanco-galdosii*. The remaining accession of *S. ×blanco-galdosii* grouped with one accession of *S. jalcae*. The Templeton test rejected the possibility of *S. peloquinianum* accessions grouping together (P = 0.0134–0.0191) as well as the possibility of *S. ×blanco-galdosii* accessions grouping together ($P < 0.0001$).

All eight accessions of *S. mochiquense* grouped together but not as a clade; the group also included all accessions of *S. chancayense* (Fig. 1). The Templeton test did not reject the possibility that all the accessions of *S. mochiquense* can group together (P = 0.5104–0.7556).

Polyploid species. — The alleles of *S. paucijugum* and *S. tuquerrense*, two of the three tetraploid representatives of clade 3, fell in two different clades. One of these clades contains only Colombian and Ecuadorian species and the alleles from both *S. paucijugum* and *S. tuquerrense* fell in a single clade, with good support, closely related to the clade that contains the majority of the accessions of *S. andreanum* (Fig. 1). The other group of alleles clustered within a poorly resolved clade of several species mainly distributed in northern Peru. The third polyploid species of clade 3, *S. solisii*, showed only two alleles; one allele was resolved in a clade with *S. paucijugum* and *S. andreanum* alleles and the other allele was resolved in a clade with alleles from *S. tuquerrense*. The possibility of both alleles clustering together was rejected by the Templeton test (P = 0.0013–0.003).

Trait reconstruction. — Tuber type (Fig. 2) and leaf texture (Fig. 3) were reconstructed on the phylogeny because states of these characters (moniliform tubers, coriaceous leaves), are commonly said to define members of ser. *Piurana*. These analyses suggest that the ancestors of clade 3 have terminal tubers. Moniliform tubers were gained in the ancestor of the rest of the clade 3 species; however, successive transitions back to the ancestral character happened in several taxa. Leaf morphology revealed a similar pattern of evolution as the moniliform tubers, except that the transitions back to the ancestral character (chartaceous leaves) were fewer than for tubers.

■ DISCUSSION

Monophyly of an expanded *Solanum* ser. *Piurana*. — The present phylogeny based on five COSII supported the three-clade structure within sect. *Petota* shown by other nuclear markers (Rodríguez & Spooner, 2009; Spooner & al., 2009). Clade 1+2 (relative to the plastid phylogeny) was basal to the sister clades 3 and 4, as was previously found by Rodríguez & al. (2009) as the dominant history using COSII markers, but not with nitrate reductase that showed clades 1+2 and 4 as sisters and clade 3 as basal to them (Rodríguez & Spooner, 2009). In contrast to all the results mentioned above, Jacobs & al. (2008) found little resolution for the South American species of sect. *Petota* using AFLP markers although they found a "Piurana Group".

Clade 3 contains species previously considered as members of ser. *Piurana* by different taxonomic treatments, but also contains species considered to belong to seven series (*S.* sers. *Conicibaccata*, *Cuneoalata*, *Ingaefolia*, *Olmosiana*, *Simplicissima*, *Tuberosa*, *Yungasensa*) suggesting that most of the series that Hawkes (1990) and other potato taxonomists defined are not supported as natural groups. The monophyly of clade 3 supports the idea of an enlarged ser. *Piurana*, relative to previous definitions of this series made by Correll (1962), Hawkes (1990) and Ochoa (1999). The relationship of members of clade 3 is also supported by the presence of moniliform tubers and coriaceous leaves among the majority of its members with few exceptions like *S. acroscopicum*, *S. huancabambense*, *S. immite*, *S. multiinterruptum and S. trinitense* that do not present either of these two characters but are well supported as members of the *Piurana* clade in the present phylogeny. A possible explanation for the failure of previous taxonomic classifications based on morphology in sect. *Petota* might be caused by these characters being ignored. The placement of *S. huancabambense* as member of clade 3 is controversial as it lacks the two morphological characters discussed above; it is at the bottom of the three and the support value for clade 3 is low (Fig. 1). However when the analysis is performed in a smaller dataset including only representative accessions for clades with high support the support value (data not shown) for clade 3 including *S. huancabambense* increases to 0.95 (Bayesian Inference) and 90% (Maximum Parsimony) (Ames, 2008).

Species and species relationships within clade 3. — We present the first expanded study of ser. *Piurana* at the species level and below, using 60 species, 199 accessions (including outgroups), five COSII markers with 5342 bp aligned length, and with access to comparative morphological data for the same accessions (Ames & al., 2008). Within clade 3 it was difficult to find well supported main clades except for the very well

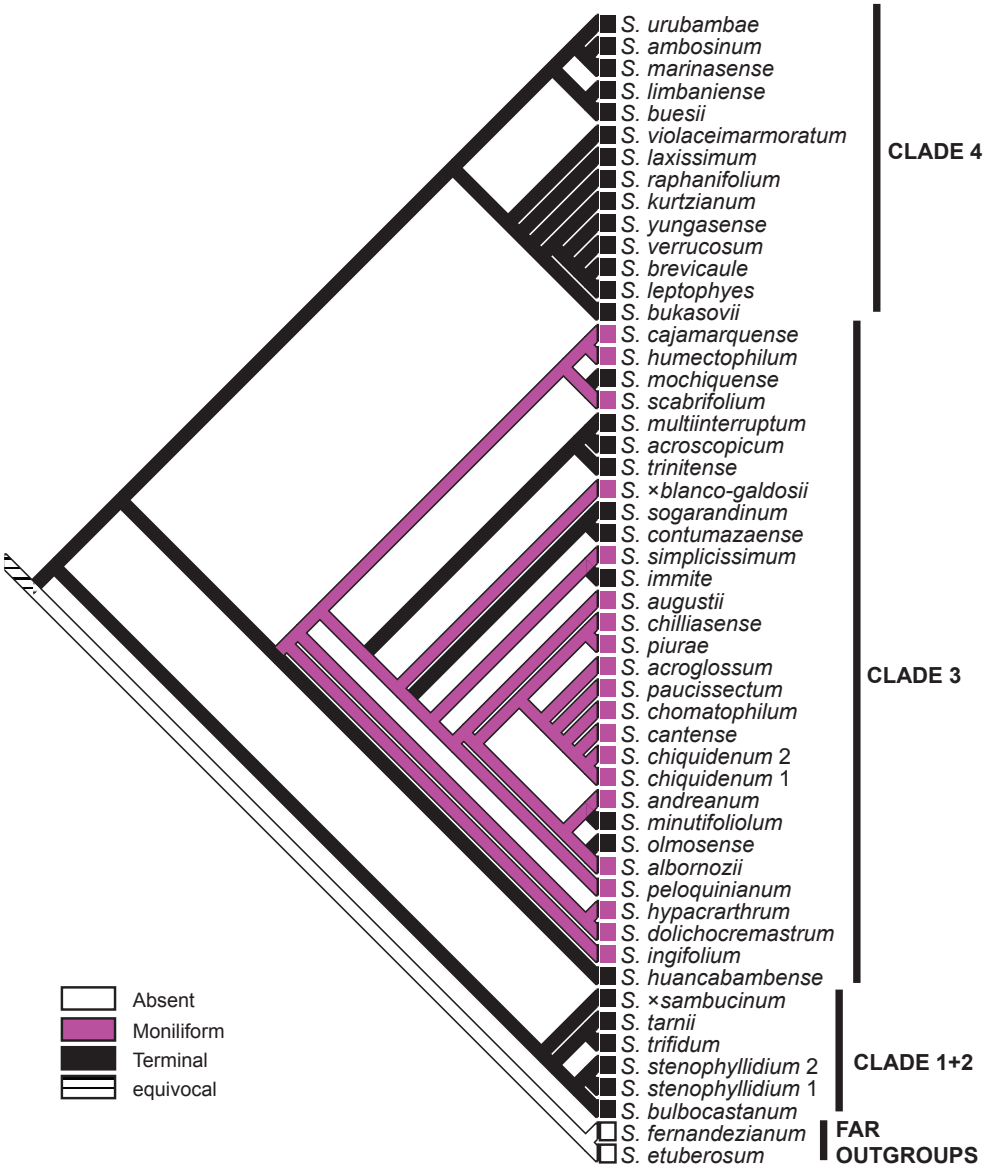

Fig. 2. Trait reconstruction for tubers. Character states are represented in different colors. Numbers after species names represent different accessions of the same species.

differentiated *S. huancabambense*, and the Ecuador-Colombia clade (Fig. 1). Other clades, which are not as well supported, showed two types of subclades; some of them containing well resolved monophyletic species and some unresolved, like the subclade containing *S. chomatophilum* (Fig. 1). The lack of resolution for this clade may be caused by rapid and recent diversification as it has been demonstrated in other Andean plant species (Hughes & Eastwood, 2006). It might be that the opportunity to colonize new habitats can also play an important role within this group, as *S. chomatophilum* is the most widely distributed species within clade 3.

Diploid species. — According to Hawkes (1990) about 12% of potato species have a hybrid origin. In the present study the majority of the species were diploid and only one of them, *S.* ×*blanco-galdosii*, was previously supported experimentally as a hybrid species through crosses in the greenhouse using *S. chomatophilum* and *S. peloquinianum* (Ochoa, 1999). The present study supports its hybrid origin, as one allele of *S.* ×*blanco-galdosii* grouped with accessions of *S. peloquinianum* and one allele grouped within a clade that included accessions of *S. jalcae* and *S. chomatophilum*.

Our analytical tools produce bifurcating trees which might be suitable to illustrate the results of the speciation process. Species are mostly considered a set of organisms that have a common gene pool held together by interbreeding. However, evolution at the species level is not always tree-like; sometimes it is network-like with a large number of reticulation events (Otto & Whitton, 2000; Linder & Rieseberg, 2004; Baum, 2007). Several of the species included in this study are supported as monophyletic and in this way reflected a bifurcating (non-hybrid) mode of speciation. However, the results also showed several species connected in a network with either accessions or alleles grouped in different clades. The high degree of morphological similarity and geographical overlapping

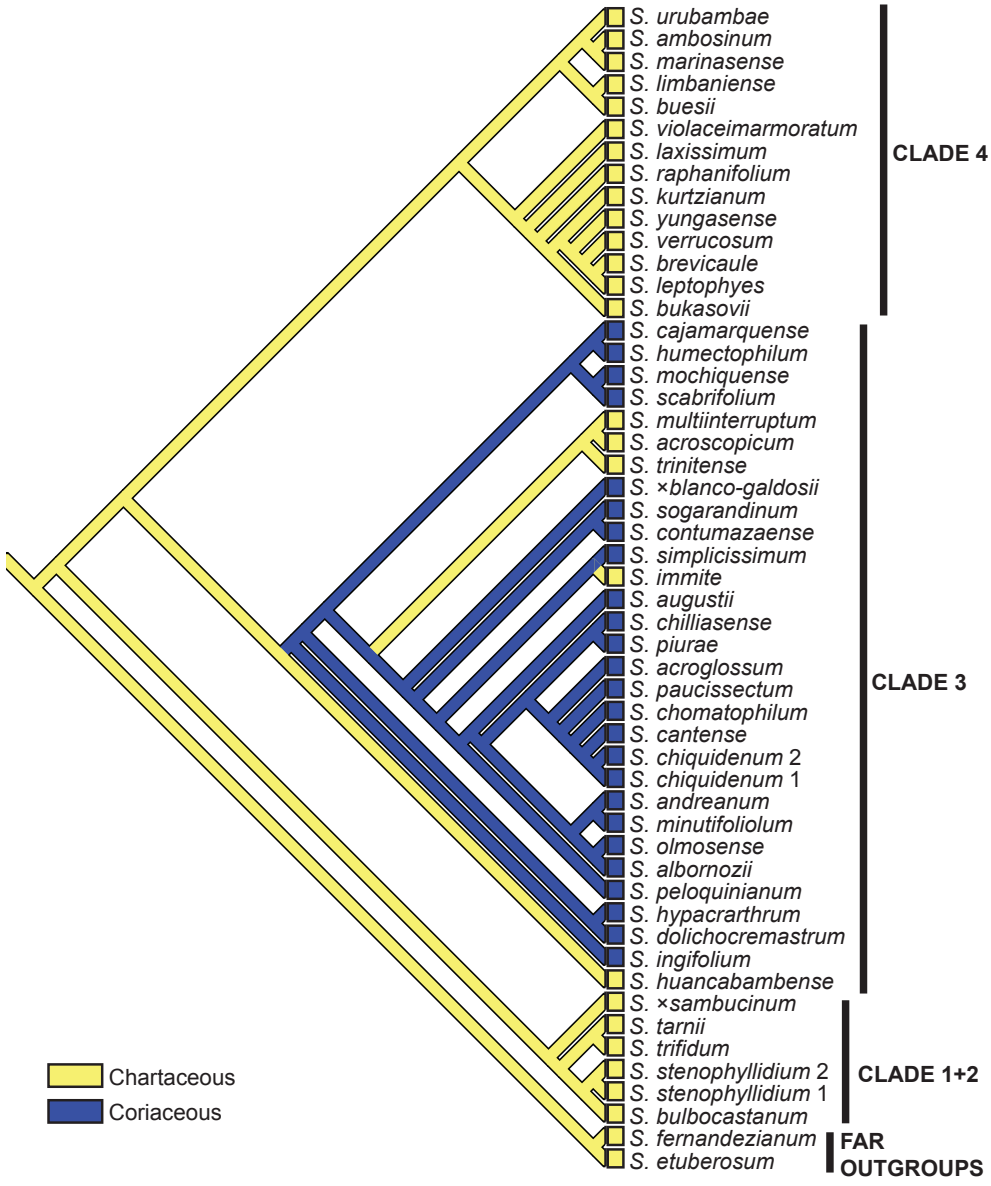

Fig. 3. Trait reconstruction for leaves. Character states are represented in different colors. Numbers after species names represent different accessions of the same species.

that allows for possible hybridization can explain some cases of alleles of *S. sogarandinum* grouping within the clade of *S. dolichocremastrum* and also *S. mochiquense* and *S. chancayense* forming a clade. The relationship between *S. jalcae* and *S. chiquidenum* is not clear; they both are well differentiated species morphologically but a 41-bp plastid deletion shared among some accessions of these species suggests gene flow between them (Ames & al., 2007). It was not possible to find any geographical or morphological association that could be related to the two clades of *S. chiquidenum*.

In general we do not consider misidentifications in the genebank accessions as having a major effect on these results because the majority of the accessions in the present study were morphologically evaluated in a previous study (Ames & al., 2008) and to the best of our knowledge they were correctly identified.

In concordance with many prior hypotheses of hybridization in sect. *Petota* (see Spooner & van den Berg, 1992 for a review), there is apparently widespread introgression and hybrid speciation in the group, as has been confirmed at the diploid level by Clausen & Spooner (1998), and at the polyploid level by Spooner & al. (2008) and Rodríguez & Spooner (2009). Backcrossing to their parents may be a force to prevent hybrids from becoming sexually distinct lineages (Linder & Rieseberg, 2004). In addition, other processes besides reticulation may be involved, such as lineage sorting or reticulation at lower levels (meiotic recombination). Clearly more research will be needed to identify hybrid species within clade 3 of sect- *Petota* but the incongruence among the COSII-independent phylogenies as shown by the ILD test might be a signal of reticulation.

Polyploid species. — Two major mechanisms have been proposed for the origin of polyploidy, one of them chromosome doubling of somatic cells and the other the union of unreduced gametes. The latter has been considered as the most common (Harlan & De Wet, 1975), and the majority of the species within sect. *Petota* form unreduced gametes (Watanabe & Peloquin, 1991). In flowering plants 2% to 4% of the speciation events might be due to polyploidization (Otto & Whitton, 2000). Polyploidy can happen either by duplication of a single genome (autopolyploidy) or when two often well differentiated genomes merge (allopolyploidy), and there is a range between strict autopolyploidy to strict allopolyploidy with intermediate types "segmental allopolyploids" (Udall & Wendel, 2006; Wendel, 2000).

Solanum ser. *Piurana* has three tetraploid species (*S. paucijugum*, *S. solisii*, *S. tuquerrense*) and all were available for study; their putative origins varied among authors. *Solanum tuquerrense* has been considered a strict allopolyploid (Matsubayashi, 1991) or a segmental allopolyploid (Gavrilenko, 2007; Watanabe & Orrillo, 1994). *Solanum paucijugum* has been considered an allopolyploid by Rodríguez & Spooner (2009), but there are no previous studies that included *S. solisii*. The present results supported *S. tuquerrense* and *S. paucijugum* to have an allopolyploid origin within clade 3 as their alleles segregated into two very well differentiated clades within clade 3. One clade contained species only from Ecuador and Colombia, and this clade included *S. andreanum* as one of the diploid potential maternal progenitors for both species, as previously suggested by a plastid DNA phylogeny (Spooner & Castillo, 1997). The other clade included species mainly distributed in Peru but *S. chomatophilum* is the likely paternal progenitor because its geographical distribution allows it to reach the area of potential hybridization with *S. andreanum* (assuming present distributions reflect past ones). *Solanum chomatophilum* and *S. andreanum* are both diploid species with EBN = 2, so theoretically they are able to hybridize and produce the two allotetraploid species *S. paucijugum* and *S. tuquerrense*.

As the alleles of both *S. tuquerrense* and *S. paucijugum* fell into the same two clades it might suggest that these species are the product of repeated polyploidization events involving the same parents, and many polyploid populations have multiple origins (Otto & Whitton, 2000) creating genetically distinct populations (Soltis & al., 2004). In the case of *S. solisii*, the alleles fell into one clade restricted to the main Ecuador-Colombia clade (Fig. 1), suggesting either an autopolyploid origin or a hybrid origin with *S. paucijugum* and *S. tuquerrense* as the potential parents. Theoretically we could expect *S. solisii* to be $2n = 4x = 48$ with EBN = 2 if *S. paucijugum* and *S. tuquerrense* were the progenitors. However, the EBN number for *S. solisii* has not been determined and chromosome counts have only been determined in one accession showing it as a tetraploid. However, the ability of *S. paucijugum* and *S. tuquerrense* to hybridize has not been well documented. In this study only one accession of *S. solisii* was included, and more research will be needed to clarify the nature of this species.

The results from the morphological study of living plants (Ames & al., 2008) and observations of herbarium specimens do not show significant differences among the three polyploid species and *S. andreanum*. Furthermore, *S. andreanum* and the polyploid species share the same geographical area (Fig. 4). This might suggest recurrent hybridizations with *S. andreanum*, at least for the sympatric populations. However, the majority of the polyploid accessions are found at higher altitudes (3100–3700 m), in contrast to *S. andreanum* which is mainly found at elevations of 2200–2900 m. This can be considered as an example of polyploids being more successful colonizing expanded habitats than their progenitors, as has been demonstrated in other plant species (Soltis & al., 2004; Otto & Whitton, 2000).

Trait evolution and the morphological definition of an expanded *Solanum* ser. *Piurana*. — The ability to tuberize is genetically controlled by at least eleven loci, all of them with small effects and highly influenced by environmental factors (Van Eck, 2007). Although tuber shape is a well studied character, the studies have been limited to the cultivated potato that has terminal (non-moniliform) tubers. In this species, terminal tuber placement seems to be regulated by a single locus, *Ro* where round tuber (*Ro_*) is dominant over long (*roro*) tuber (Van Eck, 2007). However, no genetic studies regarding moniliform vs. terminal tubers have been conducted. Although the genetics underlying the formation of different tuber types is not known, variation within populations may be due to the segregation of the character as a consequence of hybridization among species with different types of tuber placements.

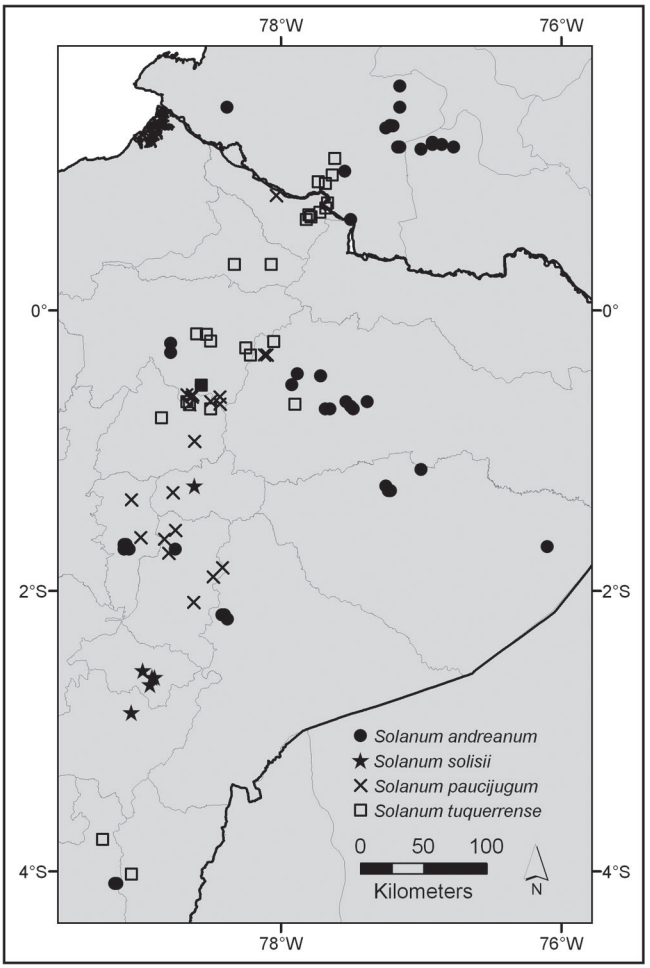

Fig. 4. Map of the geographical distribution of *S. andreanum*, *S. chomatophilum*, *S. paucijugum*, *S. solisii* and *S. tuquerrense*.

Species boundaries within clade 3. — Defining species has always been a problem and it is an impediment to studies of speciation. Species are the fundamental units of biology and are important for diverse investigations in evolutionary biology (Rieseberg & Burke, 2001; Pigliucci, 2003; Rieseberg & Willis, 2007). Species boundaries of ser. *Piurana* were investigated from two perspectives. First, we investigated morphological phenetics (Ames & al., 2008), and second, COSII-based evolutionary history. Morphological phenetics provided strong support only for four well defined groups (group 1: *S. hypacrarthrum* and *S. simplicissimum*, group 2: *S. contumazaense*, group 3: *S. peloquinianum*, group 4: all the remaining species) (Ames & al., 2008), but there is often a poor correlation between taxonomic classification and discrete phenotypic clusters, and there often are tendencies to over-differentiate species in taxonomic treatments (Rieseberg & al., 2006). Conversely, our COSII results supported some species well, despite the poor morphological support (Ames & al., 2008).

Decisions on species boundaries will be formalized in a taxonomic monograph of sect. *Petota* in preparation for northern South America. The decisions will consider data from evolutionary history (this study), morphological phenetics (Ames & al., 2008), and study of herbarium specimens, including types. As with other complicated groups experiencing the possible recent and rapid evolution and possible hybridization common in sect. *Petota* (Spooner, 2009), there is no rigid formula for such decisions.

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