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Species boundaries and interrelationships of two closely related sympatric diploid wild potato species, *Solanum astleyi* and *S. boliviense*, based on RAPDs

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Abstract The more than 200 wild and cultivated species relatives of potato (*Solanum* sect. *Petota*) present a valuable germplasm base for cultivar improvement. However, species boundaries and interrelationships within sect. *Petota* are controversial, inhibiting the efficient organization of the many germplasm collections of these species. One controversy involves questions of species boundaries and interrelationships of *S. astleyi* and *S. boliviense*. *Solanum boliviense* is narrowly endemic to two Departments in southern Bolivia, and *S. astleyi* is known only from one site entirely within the range of this species, where they co-occur. Both species are diploid and morphologically very similar. Artificial hybrids between them are fully fertile, and the species putatively hybridize naturally. These data have been interpreted to designate them as separate species or as *S. astleyi* an ecotype of *S. boliviense*. Putative progenitors of *S. astleyi* are *S. boliviense*, *S. megistacrolobum* subsp. *megistacrolobum*, and *S. megistacrolobum* subsp. *toralapanum*. We evaluated interrelationships among these species with random amplified polymorphic DNA's (RAPDs) generated for 2 accessions of *S. astleyi* and 14 accessions of *S. boliviense*. These represent the entire geographic range of the former species and nearly the entire range of the latter. We also analyzed 1 accession each of *S. acaule* subsp. *acaule*, *S. acaule* subsp. *aemulans*, *S. albicans*, *S. berthaultii*, *S. megistacrolobum* subsp. *megistacrolobum*, *S. megistacrolobum* subsp. *toralapanum*, *S. raphanifolium*, *S. sogarandinum*, and *S. sparsipilum*. Phenetic analyses of the RAPD data show *S. astleyi* and *S. boliviense* to form two distinct groups and to be more similar to each other than to any of the other species investigated, suggesting that *S. astleyi* and *S. boliviense* are sister taxa. The divergence of *S. astleyi* and *S. boliviense* relative to other species examined suggests that they are worthy of taxonomic recognition at the subspecies, rather than species level, and we propose the new combination *S. boliviense* subsp. *astleyi*.

Key words Bootstrap · UPGMA · RAPD · Section *Petota* · Taxonomy

Names are necessary to report factually and available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable

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Introduction

Solanum L. section *Petota* Dumort. contains 7 cultivated and 225 wild species according to the latest taxonomic treatment (Hawkes 1990). Of these 225 wild species 216 are tuber-bearing. A chloroplast DNA analysis (Spooner et al. 1993) supported an alternative classification (Child 1990) that removed the 9 non-tuber-bearing species from sect. *Petota*.

The tuber-bearing species are widely distributed from the southwestern United States to southern Chile. They provide a wide diversity of genes useful for cultivar improvement (Hanneman and Bamberg 1986; Ross 1986; Hanneman 1989; Hawkes and Hjerting 1989; Plaisted and Hoopes 1989; Hawkes 1990). Various genebanks worldwide maintain thousands of wild and cultivated potato accessions (Hawkes 1990). As outlined by Hawkes (1980), a refined understanding

of species boundaries and relationships are of great practical importance for breeders using germplasm resources of sect. *Petota*. However, the taxonomy of sect. *Petota* remains controversial, hindering the most efficient organization of these genetic resources (Spooner and van den Berg 1992).

One such taxonomic uncertainty involves the wild potato species *S. astleyi* and *S. boliviense*. *Solanum boliviense* is narrowly endemic to Chuquisaca and Potosí Departments in southern Bolivia. It grows from 2,600–3,750 m elevation, in rocky fields, brushy slopes, roadsides, and frequently as an agricultural weed. *Solanum astleyi* is known only from 2 sites within 0.5 km of each other in Potosí Department, at 3,280 to 3,300 m elevation, where it co-occurs with *S. boliviense* (Fig. 1; Hawkes and Hjerting 1989; Ochoa 1990).

The morphological character states distinguishing these species are outlined in the original publication of *S. astleyi* (Hawkes and Hjerting 1985a) with a later nomenclatural clarification (Hawkes and Hjerting 1985b), and from later descriptions of *S. astleyi* and *S. boliviense* in Bolivian floristic treatments (Hawkes and Hjerting 1989; Ochoa 1990). These descriptions, photographs, and drawings show them to be morphologically extremely similar, distinguished only by a complex of overlapping characters. *Solanum boliviense* is a low-growing decumbent plant (stem length to 45 cm), with leaves entire or sometimes with lateral leaf lobes that are sessile to distinctly petiolate. *Solanum astleyi* is a more erect plant (stem length 50 cm maximum), with leaves entire or sometimes with lateral leaf lobes that are always sessile. Both species have ovate leaves, but those of *S. astleyi* are sometimes narrower. The flowers and fruits are similar.

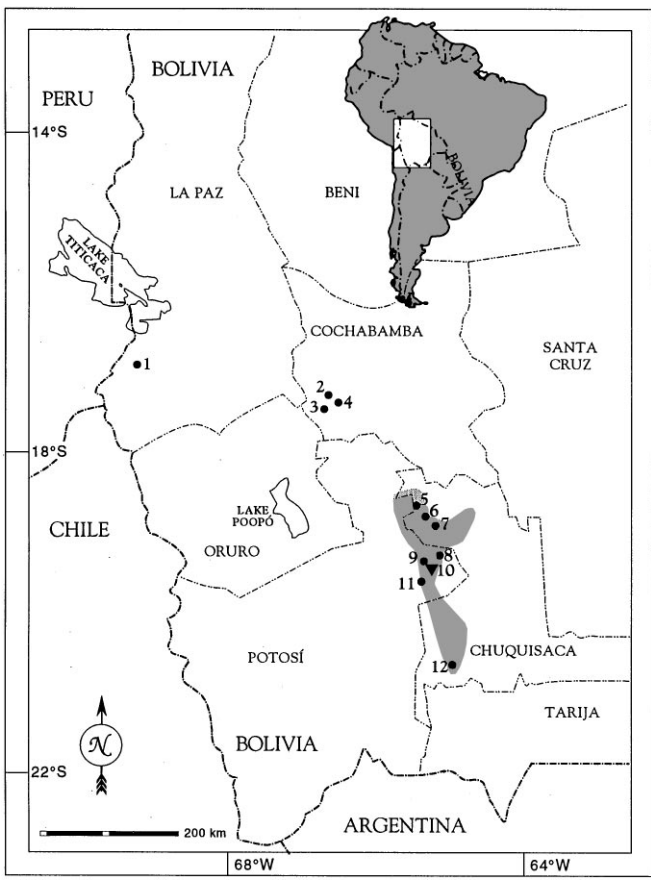

Fig. 1 Generalized distributions of the Bolivian accessions examined in this study (Table 1). The distribution of *S. boliviense* is outlined in gray and locations of populations examined are indicated; *S. astleyi* is known from only one small area within this distribution (*triangle*)

Both species are diploid ($2x = 24$), with Endosperm Balance Numbers of 2 (Hawkes 1990; Hanneman 1994). Artificial hybrids between them are fully fertile, and a putative hybrid has been collected at the locality of *S. astleyi* (Hawkes and Hjerting 1989). These morphological, distributional, and biological data have been interpreted to designate them as separate species (Hawkes and Hjerting 1989; Hawkes 1990), or as ecotypes of a single variable species (Ochoa 1990). Our field collections of five populations of *S. boliviense* in Bolivia in 1993 (*Spooner*, *van den Berg*, *García*, *Ugarte* *6524*, *6531*, *6532*, *6535*, *6612*, *6619*) further documented variability in leaf shape that showed the difficulty of using the key characters to distinguish these 2 species (Spooner et al. 1994). The collection of *S. boliviense* (*6531*) at the topotype collection of *S. astleyi* (from the location of the nomenclatural type specimen) appeared to Spooner et al. (1994) to be like other populations of *S. boliviense*, and led us, like Ochoa (1990), to suspect that the 2 species were actually conspecific.

The relationships of *S. astleyi* and *S. boliviense* to closely related species are similarly unresolved. Hawkes and Hjerting (1989) suggested that *S. astleyi* was most closely related to *S. toralapanum*, and made no statement of the relationship of *S. boliviense* to other species except to place it in ser. *Megistacroloba* (including the other Bolivian species *S. megistacrolobum* and *S. toralapanum*). Ochoa (1990) designated *S. astleyi* as an ecotype (therefore conspecific) of *S. boliviense* and inferred *S. boliviense* to be most closely related to *S. megistacrolobum*. These authors recognized *S. megistacrolobum* and *S. toralapanum* as the same species (Ochoa 1984), as varieties (Ochoa 1990), or as distinct species (Hawkes and Hjerting 1989; Hawkes 1990). Considering the morphological and molecular studies of Giannattasio and Spooner (1994a,b), we recognize these two taxa as subspecies of *S. megistacrolobum*.

The objective of the study presented here was to investigate relationships of *S. astleyi* and *S. boliviense* to each other and to closely related species based on data from random amplified polymorphic DNA's (RAPDs). RAPDs provide relatively quick, easy, and inexpensive molecular tools to investigate many biological questions. In sect. *Petota* they have been used to distinguish cultivars (Demeke et al. 1993; Mori et al. 1993; Lynch et al. 1994; Oganisyan et al. 1996), to confirm

introgression in breeding programs (Baird et al. 1992; Waugh et al. 1992; Xu et al. 1993; Takemori et al. 1994; Rasmussen and Rasmussen 1995), and for the construction of genetic linkage maps (Singsit and Ozias-Akins 1993; Quiros et al. 1993; Freyre and Douches 1994; Hosaka and Hanneman 1994; McGrath et al. 1994, 1996; Masuelli et al. 1995). More relevant to this study, RAPDs have been used to investigate species boundaries and interrelationships in sect. *Petota* (Cisneros and Quiros 1995; Spooner et al. 1996).

Materials and methods

Species

We analyzed RAPD variability among 2 accessions of *S. astleyi* and 14 accessions of *S. boliviense*, representing the entire geographic range of the former species and nearly the entire geographic range of the latter species (Fig. 1). For the 2 accessions of *S. astleyi*, two separate DNA extractions were made for each accession from separate bulks of 5 separate plants (referred to herein as replicates). We also analyzed nine other taxa of sect. *Petota* at various levels of putative divergence based on the classifications of Hawkes and Hjerting (1989), Hawkes (1990), and Ochoa (1990) (Table 1). These included all taxa of ser. *Megistacroloba* from Bolivia that are hypothesized to be related to these two species (*S. megistacrolobum* subsp. *megistacrolobum*, *S. megistacrolobum* subsp. *toralapanum*); two other taxa of ser. *Megistacroloba* from Peru (*S. raphanifolium*, *S. sogarandinum*); three taxa in ser. *Acaulia* that Hawkes and Hjerting (1989) and Ochoa (1990) believed to be closely related to ser. *Megistacroloba* (*S. acaule* subsp. *acaule*, *S. acaule* subsp. *aemulans*, *S. albicans*); and two taxa from ser. *Tuberosa* (*S. berthaultii*, *S. sparsipilum*). Some of the accessions of *S. boliviense* were collected on a recent germplasm collecting expedition to Bolivia (Spooner et al. 1994). Accessions and vouchers are deposited at the National Research Support Program-6 (NRSP-6; Bamberg et al. 1996) except for the 1993 accessions from Bolivia that are still in quarantine or are being increased. The accessions of *S. boliviense* represent the maximum geographic distribution available from the collection, and nearly the entire geographic distribution of the species. The 2 accessions of *S. astleyi* are the only accessions available and represent the entire geographic distribution of the species. We mapped all Bolivian accessions to 12 generalized areas (Fig. 1). Complete locality data are in Hawkes and Hjerting (1989), Ochoa (1990), or at NRSP-6.

DNA isolation and purification

DNA was obtained from 5–10 grams of bulked fresh leaf tissue of 5 plants per accession from 2-month-old plants. Because the goal of our study was to investigate species boundaries among *S. astleyi*, *S. boliviense*, and putative relatives, the study was designed to examine more populations rather than more individuals within populations. Preparations of total DNA followed the procedure of Doyle and Doyle (1987), substituting $6\times$CTAB for $2\times$CTAB (Smith et al. 1991), followed by purification over CsCl gradients.

RAPD primer selection, amplification, and scoring

A total of 38 10-mer RAPD primers were selected based on clearly discernible polymorphic bands from prior (Spooner et al. 1996) and unpublished studies in sect. *Petota*. These were Operon (Operon Technologies, Alemeda Calif.) RAPD primers OPA-2, OPA-4, OPA-5, OPA-12, OPA-14, OPA-15, OPA-16, OPA-18, OPAA-2, OPAA-10, OPAA-12, OPAA-14, OPAC-5, OPAE-5, OPAE-18, OPAN-15, OPAN-16, OPAO-1, OPAO-4, OPAG-2, OPAQ-1, OPB-14, OPD- 4, OPM-2, OPM-12, OPR-7, OPR-8, OPR-9, OPR-12, OPR-13, OPS-3, OPS-7, OPS-9, OPS-11, OPS-17, OPS-19, OPT-1, and OPT-8.

The RAPD reaction buffer was that of Skroch and Nienhuis (1995). RAPD reactions were performed in 10 µl volumes in a Perkin Elmer 9600 Thermal Cycler™ programmed for the cycling protocol of Skroch and Nienhuis (1995).

RAPD profiles were resolved by electrophoresis (5 V/cm) for 3 h in 1.5% Ultrapure™ (BRL) agarose gels. Gels were stained with ethidium bromide and photographed under the Eagle Eye™ Still Video System Version 1.00. Fragments ranging from 0.3 kb to 2.2 kb were scored visually for presence or absence.

Data analysis

Analyses of genetic relationships were based on Jaccard's similarity coefficient (Jaccard 1908; Gower 1972) to construct similarity and distance matrices. The pairwise comparison of 2 genotypes or accessions for a RAPD fragment has four possible outcomes: (1) amplification of the RAPD from both DNA samples, (2) amplification of the RAPD from the DNA of the first but not the second, (3) amplification from the second but not the first DNA sample, and (4) failure to amplify the RAPD from either DNA sample. Jaccard's coefficient of similarity is computed as the ratio of the number of RAPD fragments amplified from both DNA samples to the number of RAPDs for which at least 1 of the genotypes amplifies. RAPDs for which neither genotype shows amplification are appropriately not included in the calculation using RAPD data because such comparisons give no direct evidence of genome similarity or difference. However, for RAPD data, genetic similarities based on Jaccard's coefficient have been shown to be highly correlated with genetic similarities such as simple matching coefficient in potato (Spooner et al. 1996). Based on similarity and distance matrices, UPGMA (unweighted pair group method with arithmetic averaging) and MDS (multidimensional scaling) were used to describe relationships among genotypes. For MDS analysis, genetic distance was computed as 1-similarity. The UPGMA phenogram was constructed using a program written in C by one of us (PWS), and the MDS analysis was performed using SYSTAT (Wilkinson 1992).

Bootstrap probabilities

To evaluate the sensitivity of the UPGMA analysis to sampling error, we computed bootstrap probabilities for each node of the UPGMA phenogram describing the relationships among all 27 genotypes. The bootstrap analysis was performed under the assumption that the data collected in this study were collected on a random sample of RAPDs polymorphic in this germplasm. For each of 1,000 random samples of 192 bands sampled with replacement, the UPGMA analysis was repeated. For each node, the proportion of samples that supported the observed topology of the phenogram at that node was computed. A bootstrap sample supported the observed topology at a node in the observed UPGMA phenogram if the set of accessions joined by that node also joined together in the bootstrapped phenogram before any member of that set joined with any member not in that set. To evaluate the effect of sample size on bootstrap probabilities we performed the bootstrap analysis on two nodes (indicated below) individually over sample sizes of 10–200 RAPD fragments in increments of 10.

For nodes in the UPGMA phenogram CVs (coefficients of variation) for the associated genetic similarities were computed by

Table 1 Examined populations of *Solanum acaule* subsp. *acaule*, *S. acaule* subsp. *aemulans*, *S. albicans*, *S. astleyi*, *S. berthaultii*, *S. boliviense*, *S. megistacrolobum* subsp. *megistacrolobum*, *S. megistacrolobum* subsp. *toralapanum*, *S. raphanifolium*, *S. sogarandinum*, *S. sparsipilum*, and *S. tarijense*. See Fig. 1. for map locations of Bolivian accessions

Species	PI[a]	Map locality[b]	Series[c]	2*x*	Collector
Solanum acaule Bitter subsp. *acaule*	473485	Peru: Huánuco	*Acaulia* Juz.	48	*Ochoa 5035*
S. acaule subsp. *aemulans* (Bitter & Wittm.) Hawkes & Hjert.	472793	Argentina: Jujuy	*Acaulia*	48	*Okada 6007*
S. albicans (Ochoa) Ochoa	266381	Peru: Cajamarca	*Acaulia*	72	*Correll P.863*
S. astleyi Hawkes & Hjert. 1	545959	10	*Megistacroloba* Cárdenas & Hawkes	24	*Hoopes et al. 277*
S. astleyi 2	545848	10		24	*Hoopes et al. 126*
S. berthaultii Hawkes	310927	2	*Tuberosa* (Rydb.) Hawkes	24	*Ochoa s. n.*
S. boliviense Dunal 1		5	*Megistacroloba*	24	*Spooner et al. 6524*
S. boliviense 2	265860	6		24	*EBS 1795*[c]
S. boliviense 3	265861	6		24	*EBS 1847*
S. boliviense 4	498215	7		24	*Ochoa 11929*
S. boliviense 5	545963	8		24	*Hoopes et al. 275*
S. boliviense 6	545889	8		24	*Hoopes et al. 127*
S. boliviense 7	545964	8		24	*Hoopes et al. 276B*
S. boliviense 8	545965	8		24	*Hoopes et al. 278*
S. boliviense 9		9			*Spooner et al. 6535*
S. boliviense 10		10			*Spooner et al. 6531*
S. boliviense 11		11			*Spooner et al. 6619*
S. boliviense 12		12			*Spooner et al. 6612*
S. boliviense 13	310975	Bolivia		24	*Alandia 64–7*
S. boliviense 14	310974	Bolivia		24	*Alandia 64–6*
S. megistacrolobum Bitter subsp. *megistacrolobum*	473360	1	*Megistacroloba*	24	*Hawkes et al. 4853*
S. megistacrolobum subsp. *toralapanum* R. B. Giannattasio & D. M. Spooner	545927	4	*Megistacroloba*	24	*Hoopes et al. 143*
S. raphanifolium Cárdenas & Hawkes	473369	Peru: Cuzco	*Megistacroloba*	24	*Hawkes et al. 5138*
S. sogarandinum Ochoa	230510	Peru: La Libertad	*Megistacroloba*	24	*Ochoa 1450*
S. sparsipilum (Bitter) Juz. & Bukasov	473503	3	*Tuberosa*	24	*Astley 12*

[a] USDA plant introduction numbers (see Bamberg et al. 1996). Those accessions of *S. boliviense* without PI numbers will be available for distribution after increases at NRSP-6

[b] Numbers refer to Bolivian localities mapped on Fig. 1. Detailed locality data available from Hawkes and Hjerting (1989), Ochoa (1990), and NRSP-6

[c] Series classifications after Hawkes and Hjerting (1989), Hawkes (1990), and Ochoa (1990)

bootstrapping. Bootstrap samples of 192 RAPD bands were drawn with replacement from the RAPD data set, and the genetic similarity defining each node was computed as the average genetic similarity between the sets of accessions joined by that node. The variance of genetic similarities among bootstrap samples was then used to compute the coefficient of variation associated with each node. In addition, for two nodes (identified below), CVs were computed for sample sizes from 10 to 200 in steps of 10. Bootstrap probabilities and CVs for the UPGMA phenogram were computed using programs written in the C programming language by PWS.

Results

Thirty-eight RAPD primers were used to generate 192 RAPD bands polymorphic among the 27 genotypes in this study, with 3.3% missing data. A reduced data set consisting of bands polymorphic among the 2 accessions of *S. astleyi* (including replicates) and the 14 accessions of *S. boliviense* consisted of 78 polymorphic bands with 4.3% missing data.

The UPGMA phenogram of all taxa (Fig. 2) shows the 2 accessions of *S. astleyi* and the 14 accessions of *S. boliviense* forming separate clusters. In addition, these species cluster closer to each other than to any other species. Also, the replicates of each of the 2 accessions of *S. astleyi* clustered together. The 2 subspecies of *S. megistacrolobum* (ser. *Megistacroloba*) clustered together and based on the UPGMA phenogram were the most closely related taxa to *S. astleyi* and *S. boliviense*. The results also show a smaller difference between *S. astleyi* and *S. boliviense* than between subspecies within *S. megistacrolobum*, but a greater difference than between subspecies of *S. acaule*. Twenty RAPD fragments only occurred within *S. boliviense* or *S. astleyi*, including 5 fragments that were unique to *S. astleyi*, and 9 that were unique to *S. boliviense*.

For the UPGMA phenogram, bootstrap probabilities, expressed as a percentage of bootstrap samples supporting the topology at each node, varied from 7.1% to 99.5% (Fig. 2). The topology at five nodes was

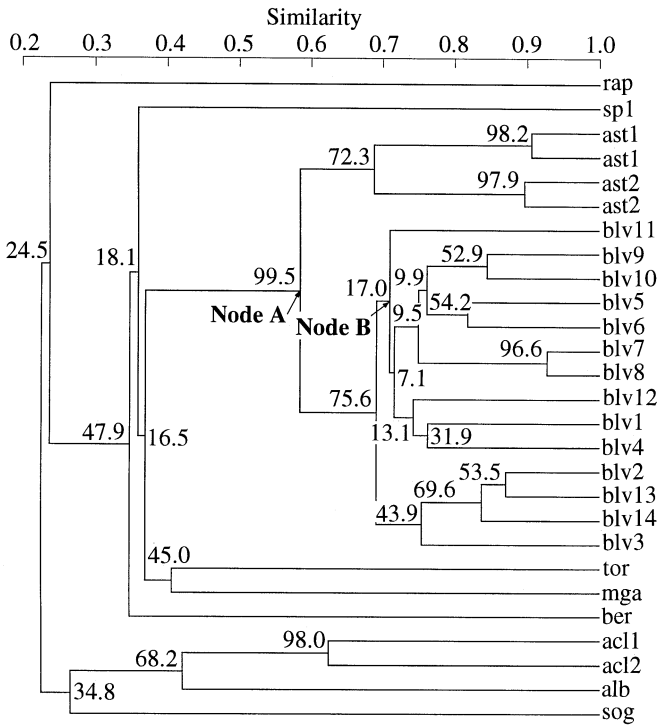

Fig. 2 UPGMA phenogram of all taxa based on 192 polymorphic RAPD fragments, with the bootstrap values supporting each node. Nodes A and B are discussed in the text

supported by more than 95% of bootstrap samples. The two nodes joining the two replicates within *S. astleyi* and the node joining *S. astleyi* to *S. boliviense* were all supported with bootstrap probabilities of 98–100%. In addition, a bootstrap probability of 98% indicates that the data strongly support the nodes joining the 2 subspecies within *S. acaule*. Support at a lower level occurred for other nodes in the UPGMA phenogram important to this study. The node joining the two *S. astleyi* accessions was supported with a bootstrap probability of only 72% while the node joining *S. boliviense* accessions was supported with a bootstrap probability of 76%.

The MDS analysis of all taxa (Fig. 3) provided a similar result to the UPGMA analysis in showing *S. astleyi* and *S. boliviense* to form taxon-specific clusters and to be more similar to each other than to other examined species. The 4 species clustering most closely to *S. astleyi* and *S. boliviense* in the UPGMA phenogram (*S. megistacrolobum* subsp. *megistacrolobum*, *S. megistacrolobum* subsp. *toralapanum*, *S. berthaultii*, *S. sparsipilum*) were also the closest taxa to *S. astleyi* and *S. boliviense* on the MDS plot. Like UPGMA, MDS also showed little variation within *S. astleyi* and *S. boliviense*, relative to the wider variation among the other taxa examined.

Bootstrap probabilities and CVs were computed for all nodes in the UPGMA phenogram using samples of 192 bands. CVs ranged from 3.6% to 16.8% with a mean of 7.9% while bootstrap probabilities varied from 7.1% to 99.5% with a mean of 50.2%. Individual nodes A and B (Fig. 2) were studied further to understand more clearly the effect of sample size on the associated CVs and bootstrap probabilities. As expected, CVs decreased and bootstrap probabilities increased with increasing band sample size (Fig. 4a,b). However, although CVs associated with each node decreased at a similar rate for different nodes, the bootstrap probabilities were very different. At node A the bootstrap probability quickly increases to over 95% for a sample size of only 100 RAPD bands. However at node B the bootstrap probability remains low, reaching a maximum of less then 20% for a sample size of 200. Overall, a non-significant correlation of -0.28 ($P > 0.05$) was observed for bootstrap probabilities and CVs computed for all nodes of the UPGMA phenogram.

Discussion

Bootstrap analysis of UPGMA phenogram

Bootstrap probabilities computed for nodes of the UPGMA phenogram measure the stability of molecular marker-determined genetic relationships over samples (Highton 1993). Under the assumption that the RAPD data collected in this study are a random sample, representative of all RAPDs polymorphic in the germplasm examined, a high bootstrap probability indicates that a different sample of RAPD loci would be unlikely to give a different result. Thus, the high bootstrap probability observed for node A in the UPGMA phenogram gives strong statistical support to the conclusion that *S. astleyi* and *S. boliviense* are more closely related to each other than to any other taxa in the study. The results of the bootstrap analysis also confirm that although *S. astleyi* and *S. boliviense* form a well-defined group (99.5% bootstrap probability), it is harder to distinguish them from each other (bootstrap probabilities of 72.3% and 75.6%). Our results are in agreement with Highton (1993) in indicating the sensitivity of UPGMA analysis to marker locus sampling effects and the difficulty of reliably determining genetic relationships among closely related taxa.

In previous studies the decrease of sampling variance and associated CVs of genetic similarity estimates with increasing marker sample size was used to evaluate the ability of a particular data set or a particular type of data to reliably determine genetic relationships among genotypes (Thormann et al. 1994; Spooner et al. 1996). However, in this study, despite low CVs at many nodes in the UPGMA phenogram indicating relatively low variances of the associated genetic similarities, low bootstrap probabilities indicate that relationships

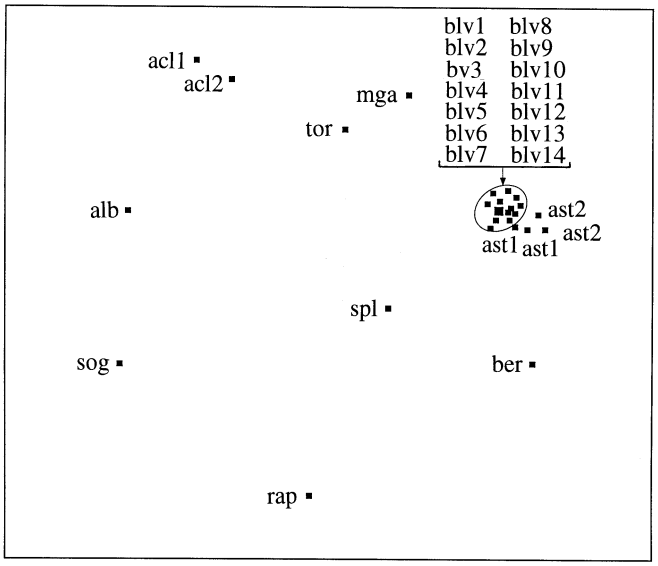

Fig. 3 MDS plot based on first two MDS coordinates of genetic distances of 192 polymorphic RAPD fragments of the 25 accessions examined in this study. Taxon abbreviations follow Hawkes (1990): *acl S. acaule* subsp. *acaule*, *aem S. acaule* subsp. *aemulans*, *alb S. aemulans*, *ast S. astleyi*, *blv S. boliviense*, *ber S. berthaultii*, *mga S. megistacrolobum* subsp. *megistacrolobum*, *tor S. megistacrolobum* subsp. *toralapanum*, *rap S. raphanifolium*, *sog S. sogarandinum*, *spl S. sparsipilum*

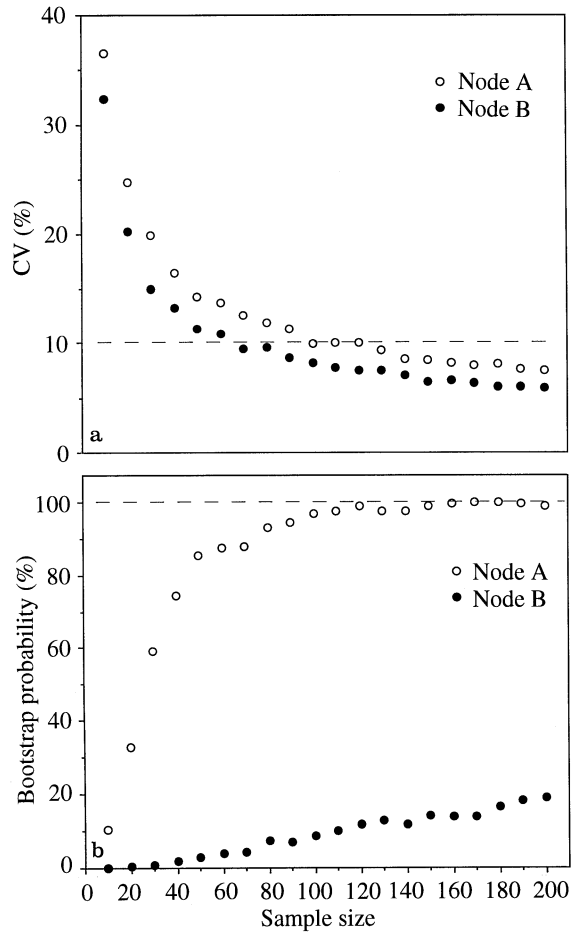

Fig. 4a, b Relationship between the number of RAPD fragments sampled and the precision of estimated genetic relationships for *S. astleyi* and *S. boliviense*. **a** The relationship between the coefficient of variation for UPGMA nodes A and B (Fig. 2) and the number of RAPD fragments used to estimate genetic similarities. **b** The relationship between the proportion of bootstrap samples supporting nodes A and B (Fig. 2) and the number of RAPD markers used in the analysis. Bootstrap probabilities and CVs are based on 1,000 samples for each sample size

among many taxa are unresolved (Figs. 2, 4). Although variances of genetic similarity estimates decrease, bootstrap probabilities for individual nodes may increase at very different rates or not at all with increasing sample size (this study; Highton 1993). The correlation of bootstrap probabilities with CVs of genetic similarity estimates, over all nodes in the UPGMA phenogram, was non-significant. These results indicate that the sampling variances or the associated CVs of genetic similarity estimates should not be used to evaluate the reliability of the results of molecular marker-based germplasm organization studies.

Species boundaries and systematic relationships of *S. astleyi* and *S. boliviense*

Phenetic analyses of morphological data in sect. *Petota* have demonstrated the extreme similarity of related taxa. These taxa often possess overlapping ranges of morphological character states, none of which is species-specific, as in the case of *S. astleyi* and *S. boliviense*. These two taxa can be distinguished only by using computer-assisted multivariate techniques or by experience, "intuitive multivariate techniques". This pattern of widely overlapping character state variability defining traditionally recognized species has been demonstrated in other wild potato species (e.g., Giannattasio and Spooner 1994a; Spooner et al. 1995; van den Berg et al. 1996). A problem in such species definitions is that there is no easy formula to distinguish taxa in traditional ways (as taxonomic keys), and different taxonomists in sect. *Petota* often differ widely in interpretations of identical collections (Spooner and van den Berg 1992; Spooner et al. 1994).

These studies demonstrate the difficulty of constructing taxonomic keys to identify taxa within sect. *Petota*. RAPD data have the advantage of supplementing morphological data at low taxonomic levels because they produce large numbers of discrete and potentially taxon-specific characters. For interspecific comparisons, comigrating RAPDs amplified by the same primer may not always represent homologous sequences (Smith et al. 1994; Thormann et al. 1994; Rieseberg 1996). However, the problem of homology of

comigrating fragments is a function of taxonomic distance (Thormann et al. 1994; Rieseberg 1996). RAPDs have been shown to be useful as taxonomic markers for closely related species through concordant results using other molecular marker systems for closely related taxa elsewhere in sect. *Petota* (Cisneros and Quiros 1995; Spooner et al. 1996). In addition, the results of the UPGMA analysis in this study are consistent with the known close relationships of accessions within *S. astleyi* and *S. boliviense*, and the subspecies of *S. acaule* and *S. megistacrolobum*.

Our study was stimulated by the alternative hypotheses of *S. astleyi* and *S. boliviense* as separate species (Hawkes and Hjerting 1989; Hawkes 1990) or as a single variable species (Ochoa 1990), and by the alternative hypotheses of relationships of *S. astleyi* to other species (Hawkes and Hjerting 1989; Ochoa 1990). Phenetic analyses of the RAPD data show *S. astleyi* and *S. boliviense* to form two distinct phenetic groups and to be more closely related to each other than to other species in this study.

Numerous collecting expeditions to Bolivia have searched for *S. astleyi*, and it apparently is very rare, having only been collected in the immediate area of the type collection (Hawkes and Hjerting 1985a,b, 1989; Ochoa 1990; Spooner et al. 1994). However, collection *6531*, collected at the type locality of *S. astleyi*, clustered with other populations of *S. boliviense*, not *S. astleyi*. Apparently, we overlooked *S. astleyi* at this locality where it was originally found, or it has subsequently disappeared.

The morphological and genetic similarity of these taxa, their interfertility, and sympatric distributions are consistent with a hypothesis that *S. astleyi* and *S. boliviense* are sister taxa. The RAPD data show *S. astleyi* and *S. boliviense* to form two distinct groups and to be more similar to each other than either one is to any of the other species investigated. The divergence of *S. astleyi* and *S. boliviense* from each other suggests that they are worthy of taxonomic recognition at the variety or subspecies level rather than species level. This taxonomic interpretation is intermediate to the species designation of Hawkes and Hjerting (1989) and their designation as the same species by Ochoa (1990). We agree with Stuessy (1990) and Hamilton and Reichard (1992) who suggest that subspecies should be used when only one rank below species is needed. We therefore propose the new combination *S. boliviense* subsp. *astleyi* to provide a taxonomic interpretation to our new results.

Solanum boliviense Dunal subsp. *astleyi* (Hawkes and Hjert.) D.M. Spooner, M. Ugarte, and P.W. Skroch, comb. et stat. nov. – *S. astleyi* Hawkes and Hjert., Bot J Linn Soc 91:445. 1985 [this publication properly designates the holotype and fulfills criteria for valid publication; it refers to an earlier publication (Bot J Linn Soc 90:105–112. 1985) with the description of the species and photo of the holotype but no designation of a holotype]. – TYPE: Bolivia, Department Potosí, Province Saavedra, 11.5 km along the road to Esquire from the turning point on the Betanzos to Turuchipa road, alt. 3,300 m; March 3, 1980; *W. Hondelmann, D. Astley, and A. Moreira 208* [K, (photo: Bot J Linn Soc 90: p. 108. 1985!), (drawing: Hawkes JG, Hjerting JP 1989: The potatoes of Bolivia: their breeding value and evolutionary relationships. Oxford University Press, Oxford, p 206!)].

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