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ORIGINAL ARTICLE

A morphological study of species boundaries of the wild potato *Solanum brevicaule* complex: replicated field trials in Peru

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Abstract The *Solanum brevicaule* complex contains about 20 species of diploids ($2n = 2x = 24$), tetraploids ($2n = 4x = 48$) and hexaploids ($2n = 6x = 72$), distributed from central Peru south to northwestern Argentina. The complex is defined entirely by morphological similarity of its constituent members, which are very similar to each other and to some landraces of the cultivated potato, *Solanum tuberosum*. Conflicting taxonomic treatments are common among authors. Species boundaries within the complex have been studied with morphological phenetics from germplasm accessions planted in a field plot in the north central US, and with molecular marker data from RAPDs, low-copy nuclear RFLPs, and AFLPs. The present study compares these results with an additional replicated morphological study of the same germplasm accessions in a greenhouse environment in the high Andes of central Peru. The results support extensive reduction of species in the complex.

Keywords Phenetics · Replicated field trials · *Solanum brevicaule* complex · *Solanum* sect. *Petota*

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Introduction

The potatoes and their wild relatives, *Solanum* L. sect. *Petota* Dumort., grow from the southwestern US to Argentina and Chile, and in the east of South America in Paraguay, Brazil and Uruguay. The latest comprehensive taxonomic treatment by Hawkes (1990) included 232 tuber-bearing and non-tuber-bearing species. Molecular studies have redefined sect. *Petota* to be entirely tuber-bearing (Spooner et al. 1993) and molecular and morphological studies are reducing the number of species to its current number of 188 species, but with predictions that this number will continue to decrease (Spooner and Salas 2006).

The *Solanum brevicaule* complex (see Table 1 for author names) is a group within sect. *Petota* containing about 20 species, with about 30 taxa including their subspecies and varieties. With synonyms, the complex includes 72 names (see Table 1 of Van den Berg et al. 1998), containing fully 14% of the estimated 531 basionyms in sect. *Petota* (Spooner and van den Berg 1992). The complex is defined entirely by morphological similarity of its constituent members that are distributed from Peru south to northwestern Argentina. They are very similar to each other and difficult to distinguish from some landraces of the cultivated potato, *Solanum tuberosum*. All members of the complex: (1) have pinnately dissected leaves, (2) have rotate to rotate-pentagonal corollas, (3)

have round fruits, (4) are largely sexually compatible, (5) are hypothesized to form occasional natural hybrid swarms, (6) have Endosperm Balance Numbers (EBN, a phenomenon of sexual compatibility based on ratios of maternal/paternal genomes in the endosperm) matching their ploidy levels (except the hexaploids that are 4 EBN), and (7) are frequently confused in the literature, in herbaria, and in germplasm collections (Van den Berg et al. 1998). For example, a comparison of independent identifications of identical collection numbers of members of the *S. brevicaule* complex from the treatments of the wild potatoes of Bolivia by Hawkes and Hjerting (1989) and Ochoa (1990) showed them to differ in 38% of the cases (Spooner et al. 1994). They vary in ploidy from diploid ($2n = 2x = 24$), to tetraploid ($2n = 4x = 48$), to hexaploid ($2n = 6x = 72$) (Hijmans et al. 2007).

The species boundaries of the complex were studied with morphological phenetics from germplasm accessions planted in a field plot in Wisconsin by Van den Berg et al. (1998), with low-copy nuclear RFLPs and RAPDs by Miller and Spooner (1999), and with AFLPs by Spooner et al. (2005). All data were concordant in showing (1) there are two groups of species, one from Peru and northwestern Bolivia (the "northern" group); and a second from northwestern Bolivia to northwestern Argentina (the "southern" group), (2) the cultivated accessions formed a monophyletic group within the northern *S. brevicaule* group, supporting southern Peru as the origin of the cultivated potato, (3) there were no species-specific morphological characters, even when the northern and southern members of the complex were treated as two species, and (4) as a result, any species that may be valid would be distinguished only by the use of a complex of character states overlapping in range (polythetic support).

The morphological study of Van den Berg et al. (1998) was conducted in a field in the northern US (45°N, 180 m over sea level). The present study analyzes data from a replicated morphological study of the same germplasm accessions in a very different environment of a greenhouse in the central Peruvian Andes. The purpose of the present study is to gain morphological data from a site in Peru more typical than the US study regarding altitude and day length, and to compare the results to the prior US morphological data sets and molecular data sets. These prior studies indicated the need to reduce the number of species in the complex. Because the *S. brevicaule* complex contains so many species used in cultivar development, we wish to have as many appropriate data available before making this decision. All species are members of plastid "clade 4" of Spooner and Castillo (1997), that also includes members outside of the complex, but morphological and molecular marker data (RAPDs, nuclear RFLPs, and AFLPs) suggest that the group is not monophyletic within this clade. The goal of the present research is to better understand morphological support for species boundaries within the complex, for a monograph of these species that will include additional herbarium and DNA sequence data.

Materials and methods

Plant material

A total of 137 accessions from 34 different taxa of the *S. brevicaule* complex and phenetically distinctive species, not part of the complex, for comparison were assessed in the morphological analyses (Table 1). These represent many of the same accessions of those studied with morphology in the US (Van den Berg et al. 1998), RAPDs and nuclear RFLPs (Miller and Spooner 1999) and AFLPs (Spooner et al. 2005). The accessions came from the US Potato Genebank in Sturgeon Bay, WI (http://www.ars-grin.gov/nr6/). Not all the species in the replicated studies had the same number of accessions due to their rarity, restricted geographical distribution, and different survival at the experimental sites. All accessions were mapped by Van den Berg et al. (1998) to 59 generalized geographic areas (Fig. 1). Five plants per accession were planted for each of two replicates and three plants were measured for each replicate for a total of six individuals per accession that were measured. Plants were grouped by accession but the accessions were randomly mixed.

Growth conditions

Accessions were grown at the International Potato Center (CIP) Huancayo Research Station in the central Peruvian Andes (3,200 m above sea level, 12°S, 75°W). Seeds were planted in September 2001, and seedlings were transplanted into 8 in. pots in organic soil and grown in greenhouses. Two prior studies attempted to grow these plants in a field plot at Huancayo but failed for various reasons, so we grew them in a greenhouse setting where CIP has experience growing and maintaining plants. The plants were watered daily and treated with insecticides and fertilized as needed. Measurements were made in February to March 2002.

Data measurement

A total of 52 characters (see Table 3 of Van den Berg et al. 1998) were evaluated. Leaf measurements were taken from the middle leaf of each plant at the initiation of or during flowering. Colors were assessed using the RHS Colour Chart (Royal Horticultural Society 2001). To insure

Table 1 The 137 accessions of 34 taxa examined in the morphological study of the *S. brevicaule* complex in Peru

Solanum abancayense Ochoa[a]	442700[b], 16[c]; 458403, 16 ; 458404, 16; 558032, 29
S. acroscopicum Ochoa	230495, 27; 365314, 21; 365315, 10
S. ambosinum Ochoa	365362, 2; 498209, 3; 498210, 3; 498211, 3; 498212, 3; 498213, 3
S. avilesii Hawkes and Hjert.	498091, 36; 498092, 36; 498093, 36
S. brevicaule Bitter	473378, 34; 498111, 34; 498112, 34; 498114, 34; 498115, 34; 498218, 34; 545967, 34; 545969, 34; 545970, 29; 545971, 34
S. bukasovii Juz.	365304, 7; 414155, 15; 473494, 10; 568933, 24; 568954, 24
S. canasense Hawkes	210035, 18; 246533, 18; 265863, 25; 265865, 32; 458376, 25; 473348, 18; 473355, 12
S. candolleanum P. Berthault	498226, 29; 498227, 28; 498313, 29; 545972, 29
S. curtilobum Juz. and Bukasov	186181, *X*
S. gourlayi Hawkes subsp. *gourlayi 2x*	414145, 49; 473011, 49; 473065, 55; 500022, 49; 537026, 40
S. gourlayi subsp. *gourlayi 4x*	442673, 48; 473007, 49; 473015, 49; 558062, 56
S. gourlayi subsp. *pachytrichum* (Hawkes) Hawkes and Hjert	545865, 40; 545866, 40; 545867, 40; 545975, 44; 545976, 44
S. gourlayi subsp. *pachytrichum*	545977, 44; 545978, 44
S. gourlayi subsp. *vidaurrei* Cárdenas) Hawkes and Hjert	472991, 49; 472995, 52; 472998, 52; 473000, 51; 498332, 52
S. hondelmannii Hawkes and Hjert	473365, 44; 498067, 44; 498071, 40; 545869, 44; 545870, 44; 545871, 44; 545872, 44; 545876, 40; 545877, 40; 545878, 40; 545879, 35
S. hoopesii Hawkes and K. A. Okada	545881, 45; 545882, 45
S. incamayoense K. A. Okada and A. M. Clausen	473066, 55. 473067, 55; 473068, 55; 473069, 55
S. leptophyes Bitter	458378, 26; 473342, 31; 473343, 31; 473445, 18; 473451, 14; 545985, 39; 545987, 39; 545992, 39; 545997, 39
S. marinasense Vargas	498254, 22; 498255. 19
S. medians Bitter	230507, 6; 265872, 6; 283081, 6; 310994, *X*; 320260, 6. 458402, 5; 473496, 6
S. multidissectum Hawkes	210043, 8; 210052, 12; 210055, 23; 473349, 18; 473352, 23; 473353, 20; 473354, 15; 498304, 20
S. oplocense Hawkes	458392, 41; 473198, 47; 498070, 42; 435079, 48; 473187, 48; 473190, 47; 473368, 42; 498068, 43; 545909, 42
S. pampasense Hawkes	275274, 12; 442697, 11; 458381, 12
S. sparsipilum (Bitter) Juz. and Bukasov	230502, 18; 246536, 18; 473384, 31; 473385, 18; 498134, 35; 498140, 34; 498284, 30; 498285, 40
S. stenophyllidium Bitter	545812, *X*; 545815, *X*; 545816, *X*; 558460, *X*
S. stenotomum Juz. and Bukasov subsp. *goniocalyx* (Juz. and Bukasov) Hawkes	195186, 12. 195188, 13; 195214, 4; 230512, 1
S. stenotomum subsp. *stenotomum*	195204, 17; 205526, 18; 205527, 4; 234012, *X*; 234015, *X*
S. stoloniferum	
S. stoloniferum Schltdl.	497998, *X*; 498238, *X*; 558395, *X*; 558396, *X*; 497995, *X*
S. sucrense	473388, 40; 545915, 45; 546008, 45
S. tuberosum subsp. *andigenum*	265882, 44; 281038, *X*; 281208, *X*
S. ugentii Hawkes and K. A. Okada	546030, 45; 546032, 45
S. vernei Bitter and Wittm. subsp. *ballsii* (Hawkes) Hawkes and Hjert	458370, 53; 473303, 49; 500070, 53
S. verrucosum Schltdl.	275256, *X*; 310966, *X*; 365404, *X*; 498010, *X*; 498061, *X*; 545745, *X*; 545747, *X*; 558482, *X*

[a] The taxon names of the *S. brevicaule* ingroup taxa remain the same as those in Van den Berg et al. (1998) and Miller and Spooner (1999) for ease of comparison of studies, but see Spooner and Salas (2006) who document changes of *S. canasense* (=*S. bukasovii*), *S. gourlayi* (=*S. leptophyes*), and Spooner et al. (2007) who place all taxa of *S. tuberosum* and *S. stenotomum* into *S. tuberosum* without subspecies. However, the names of the Central American species are changed based on Spooner et al. (2004): *S. fendleri* (=*S. stoloniferum*), *S. brachistotrichium* (=*S. stenophyllidium*)

[b] Plant introduction number from the US Germplasm System

[c] Map localities follow Fig. 1; *X* signifies localities not appearing in Fig. 1

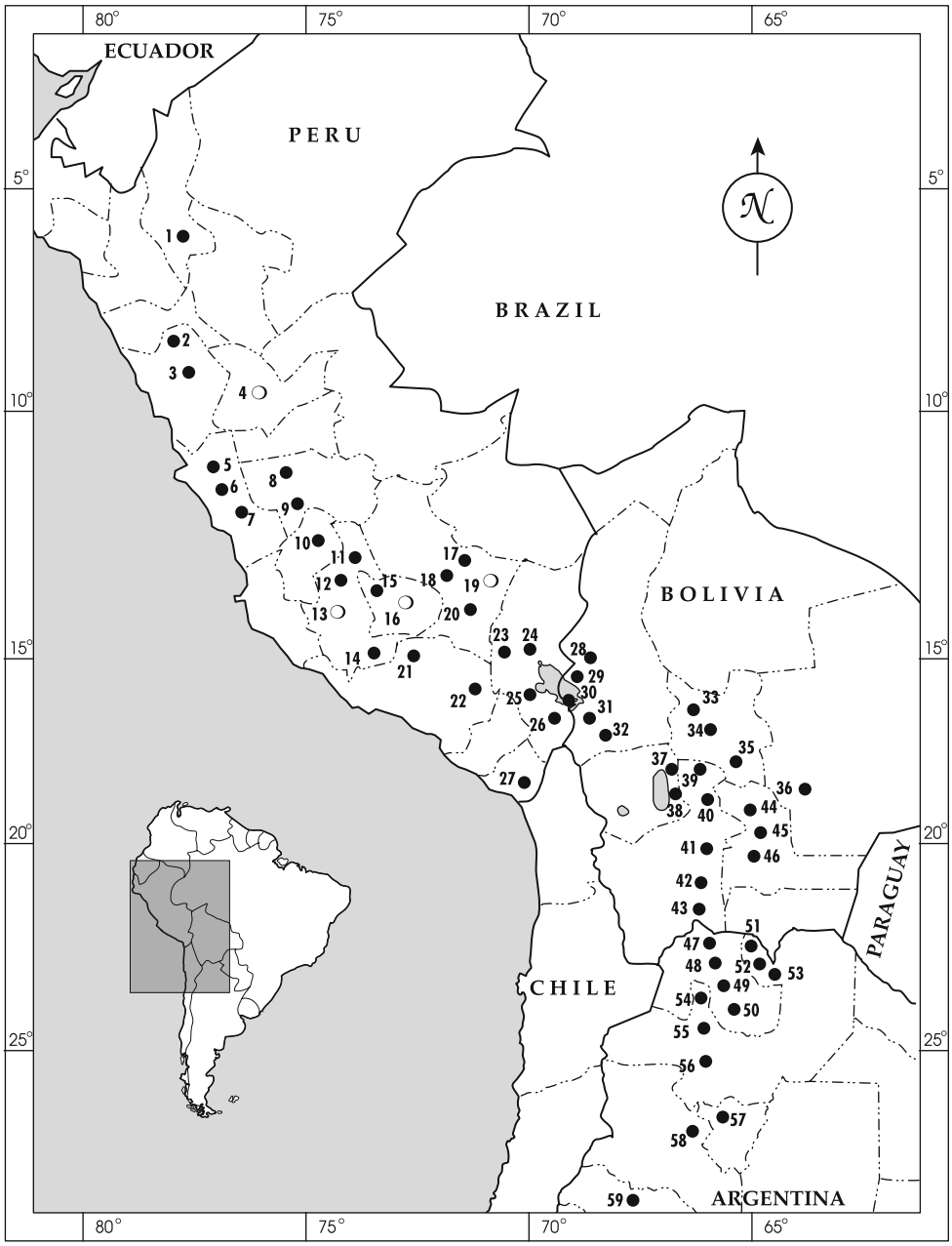

Fig. 1 Map [reproduced from van den Berg et al. (1998)] showing the 59 generalized areas of the accessions of the *S. brevicaule* complex examined in this study. *Solid circles* are areas with good locality data; *open circles* are localities that can be mapped only to department (or province). Generalized map areas correspond to those in Table 1

uniformity of measurements (i.e., measurements of pubescence, leaves, or corollas), the same person measured each character type. The same characters were measured for the prior US and the present Peru study except character 26 was not examined in Peru.

We used a Hunter Lab Color Quest 45/0 colorimeter to convert RHS colors to CIELab values, which is a color model used to describe all the colors visible to the human eye. The L^* parameter represents the lightness of the color or luminance [$L^* = 0$ (black) and $L^* = 100$ (white)], a^* the position between green (when negative) and red (when positive), and b^* the position between yellow (positive values) and blue (negative values). Therefore, color characters 50, 51, 52, 53 were scored differently in this study.

Data analysis

The study of accessions in the US (Van den Berg et al. 1998) showed principal components analysis to intermix species tremendously and only canonical discriminant analyses (CDA) to be effective to separate species and the northern and southern groups of *S. brevicaule*. Hence, we used CDA, using averages of the six plants

from each measurement as representative of the accession (thus the accession is the operational taxonomic unit, OTU). CDA attempts to portray multidimensional variation in the data set in the fewest possible dimensions, while maximizing the variation. It uses assigned groups to derive a linear combination of the variables (morphological characters) that produces the greatest separation of the groups (SAS Institute Inc. 2004). We used stepwise discriminant analyses (SDA) to determine the characters that best distinguished species in the *S. brevicaule* complex by the elimination of all accessions of the phenetically distinctive *S. stenophyllidium* (formerly *S. brachistotrichium*), *S. stoloniferum* (formerly *S. fendleri*), and *S. vernei* (referred to as the reduced data set). SDA determines (and ranks from most important to least important) the subset of the characters best discriminating groups (here species or subspecies). All analyses were done in SAS ver 9.1 (SAS Institute Inc. 2004). Based on results from the US morphological data we ran the CDA twice, once with all accessions and a second time with the reduced data set.

We further analyzed taxa from the northern and southern *S. brevicaule* complex groups separately by SDA and CDA. Within these individual studies we eliminated taxa that were (1) phenetically distinctive, (2) outgroups of the *S. brevicaule* complex, (3) cultivated species, and (4) using only a subset of the 52 characters that were shown by SDA to distinguish species. We deleted S. *curtilobum*, *S. medians*, *S. stenophyllidium*, *S. stenotomum* (both subspecies), *S. stoloniferum*, *S. tuberosum*, *S. vernei* (both subspecies), and *S. verrucosum*.

We further analysed characters separating *S. spegazzinii* in the prior US results (this species did not grow well in Peru) from the rest of the *S. brevicaule* group because it was supported as a good species by AFLP data (Spooner et al. 2005). We labeled *S. spegazzinii* as one taxon and the rest of the *S. brevicaule* complex (north and south groups treated as one) as another taxon to identify the characters that best separated these two groups, and ran CDA of these two groups.

Using procedures in NTSYS-pc ver 2.0 (Rohlf 1997) we constructed similarity matrices using the distance (DIST) procedure for the morphological data sets from the two study sites of the US and Peru, the Jaccard's procedure for the RAPD and AFLP data, and the simple matching procedure for the nuclear RFLP data. From these we determined correlation coefficients of the similarity matrices for all pair-wise comparisons using MXCOMP. We also constructed Neighbor Joining trees of all six morphological and molecular marker matrices, determined the cophenetic coefficients of these trees with COPH, and used MXCOMP to compare these cophenetic coefficients.

Results

Multivariate analyses

There were 1.9% missing averages for the Peruvian data. Because SAS eliminates entire accessions with any missing data we estimated values of missing characters from averages of other accessions of the same species. The evaluations showed the *L*a*b** color values to be highly correlated, and we selected only the parameter *a** only for analysis.

The CDA of the entire data set from the Peruvian study site is presented in Fig. 2a. It clearly separates the non-*S. brevicaule* complex species from Mexico *S. stenophyllidium* and *S. stoloniferum*. Unlike the US results, however, *S. verrucosum* and *S. vernei* are included within the cluster of the complex. It is hard to discern any species-specific clusters within this main cluster.

The CDA of the reduced data set of only members of the *S. brevicaule* complex accessions (i.e., without *S. stenophyllidium*, *S. stoloniferum*, and *S. vernei*; Fig. 2b) shows many similarities to the US results of Van den Berg et al. (1998): (1) OTUs fall along a geographical axis, with accessions from Peru and adjacent northwestern Bolivia (*S. candolleanum*) grading into accessions from Bolivia and Argentina (and *S. verrucosum* from Mexico), (2) the cultivars fall adjacent to the wild species of the northern complex, (3) although there are trends of species clustering, there is no appreciable phenetic structure separating species, (4) most (but not all) accessions of *S. oplocense* form a cluster, near accessions of *S. hondelmannii*, a species Ochoa (1990) indicated would probably be better classified as a hybrid variant of *S. oplocense*, and *S. incamayoense* and tetraploids accessions of *S. gourlayi*.

The SDA identified the following five characters to be the most important in discriminating taxa within the reduced (*S. brevicaule* complex taxa only) data set, ranked by decreasing importance: (1) number of flowers per inflorescence, (2) ratio: length of terminal leaflet/width of terminal leaflet, (3) ratio: length of third most distal lateral leaflet/length of second most distal lateral leaflet, (4) color of abaxial corolla ray, (5) length of terminal leaflet petiolule. As a point of comparison, the US data (Van den Berg et al. 1998) identified the five most important characters to distinguish species in the SDA, ranked by decreasing importance, to be: (1) width of most distal lateral leaflet, (2) density of abaxial leaf pubescence, (3) shape of terminal leaflet base, (4) plant height, and (5) diameter of style. A graphical presentation of means, ranges, and standard deviations of the characters from the Peruvian study site (not shown), like the characters from the US site (Fig. 6 of Van den Berg et al. 1998), show extensive overlap of ranges of characters among species.

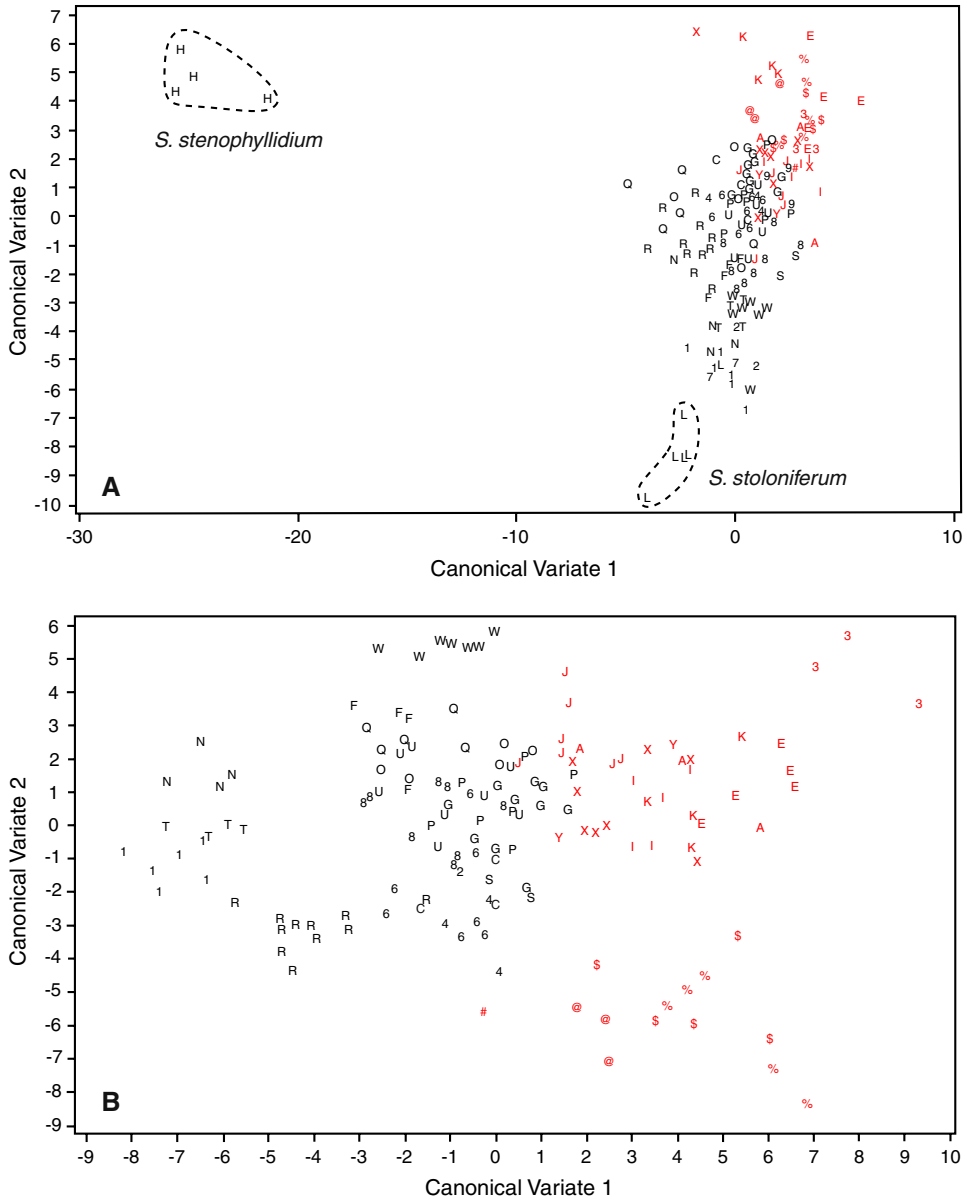

Fig. 2 Results of the study in Peru. **a** Canonical discriminant analysis of the entire data set of members of the *S. brevicaule* complex and other species in sect. *Petota*, based on 52 morphological characters. Taxon symbols are: *S. abancayense*, A; *S. acroscopicum*, C; *S. ambosinum*, E; *S. avilesii*, F; *S. brevicaule*, G; *S. stenophyllidium*, H; *S. bukasovii*, I; *S. canasense*, J; *S. candolleanum*, K; *S. fendleri* (OG), L; *S. gourlayi* (2*x*) O; *S. gourlayi* (4*x*) N; *S. gourlayi* subsp. *pachytrichum*, P; *S. gourlayi* subsp. *vidaurrei*, Q; *S. hondelmannii*, R; *S. hoopesii*, S; *S. incamayoense*, T; *S. leptophyes*, U; V; *S. medians*, W; *S. multidissectum*, X; *S. marinasense*, Y; *S. oplocense*, (4*x*) 2; *S. oplocense* (6*x*) 1; *S. pampasense*, 3; *S. sucrense*, 4; *S. sparsipilum*, 6; *S. ugentii*, 7; *S. verrucosum* (OG), 8; *S. vernei* subsp. *ballsii*, 9; *S. curtilobum*, # sign; *S. stenotomum* subsp. *stenotomum*, dollar sign; *S. stenotomum* subsp. *goniocalyx*, percent sign; *S. tuberosum* subsp. *tuberosum*, asterisk; *S. tuberosum* subsp. *andigenum*, @ sign. Outgroup taxa are delimited with a *dotted line*. **b** Canonical Discriminant Analysis of the reduced data set of members of the *S. brevicaule* complex (eliminating *S. stenophyllidium, S. stoloniferum*, and *S. vernei*), based on 52 morphological characters

Refined analysis within the northern and southern members of the *S. brevicaule* complex

SDA results of the northern and the southern components of the complex analyzed separately are presented in Table 2. These results show a range of 16–18 characters best distinguishing species in these studies, with only some of the characters similar within the two geographical groups.

The CDA results of the northern members of the *S. brevicaule* complex using only the characters from this

Table 2 Ranked characters (in decreasing importance) distinguishing taxa within the northern and southern members of the *S. brevicaule* complex in the Peruvian study as discerned by stepwise discriminate analyses

Northern group	Southern group
33	16
24	32
42	52
20	5
44	12
45	15
29	44
39	4
17	21
51	48
25	7
38	33
10	46
16	25
5	3
49	1
	35
	47

Character numbers correspond to Table 3 of Van den Berg et al. (1998)

1 Color of stem: (1) entirely green, (2) mostly green, (3) equally green and purple, (4) mostly purple, (5) entirely purple. *3* Plant height (cm) *4* Length of adaxial leaf pubescence (mm). *5* Density of adaxial leaf pubescence (number of hairs/ cm^2). *7* Density of abaxial leaf pubescence (number of hairs/cm^2). *10* Ratio: length from widest part of leaf to apex/length of leaf. *12* Number of interjected leaflets. *15* Length of terminal leaflet. *16* Ratio: length of terminal leaflet/width of terminal leaflet. *17* Ratio: length from widest part of terminal leaflet to apex/length of terminal leaflet. *20* Length of most distal lateral leaflet petiolule. *21* Angle of most distal lateral leaflet from rachis, as measured by ratio: one half of width between apices of most distal lateral leaflet pair/length of most distal lateral leaflet. *24* Ratio: length from widest part of most distal lateral leaflet to apex/length of most distal lateral leaflet. *25* Width of wing of rachis of most distal lateral leaflet. *29* Length of leaf from apex to widest point. *32* Length of peduncle. *33* Number of peduncle forks. *35* Ratio: number of flowers inflorescence/number of forks per inflorescence. *38* Length of calyx acumen. *39* Length of calyx lobe. *42* Ratio: length from center of corolla to base of corolla lobe/radius of corolla. *44* Length of anther. *45* Length of style exsertion from apex of anthers apex of stigma. *46* Diameter of style. *47* Ratio: diameter of style/diameter of stigma. *48* Length of stigma. *49* Diameter of stigma/length of stigma. *51* Color of abaxial corolla interpetolar tissue. *52* Color of adaxial corolla ray. *53* Color of abaxial corolla ray

SDA analyses are presented in Fig. 3. This analysis shows some phenetic support for *S. ambosinum*, *S. candolleanum*, and *S. pampasense*. The CDA results of the southern members of the *S. brevicaule* complex using these characters from the SDA are presented in Fig. 4. It is more difficult to discern phenetic structure from these southern complex species. It can be roughly interpreted to support

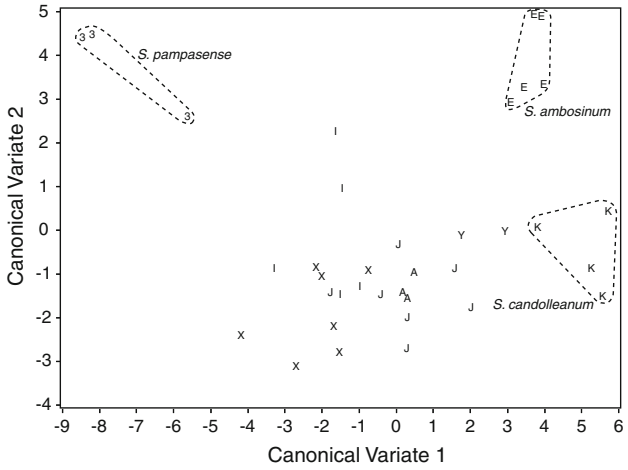

Fig. 3 Canonical discriminant analysis of the northern members of the *S. brevicaule* complex from Peru using only the characters discovered by stepwise discriminant analysis to distinguish species within these groups (Table 2)

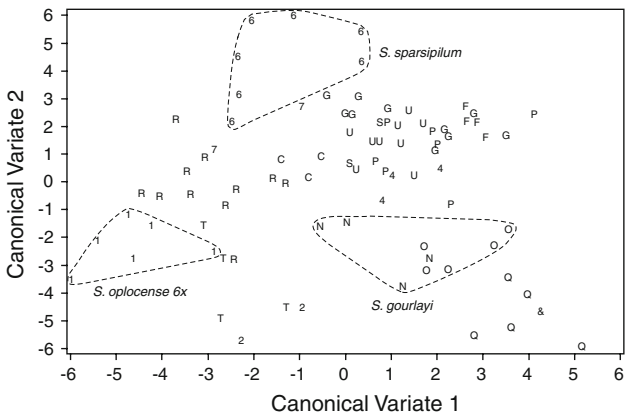

Fig. 4 Canonical discriminant analysis of the southern members of the *S. brevicaule* complex from Peru using only the characters discovered by stepwise discriminant analysis to distinguish species within these groups (Table 2)

S. gourlayi diploids and tetraploids, *S. oplocense* hexaploids (close to *S. hondelmannii* in the US and Peruvian study), and *S. sparsipilum*.

Refined analysis of *S. spegazzinii*

AFLP data (Spooner et al. 2005), but not morphological data, supported *S. spegazzinii* as a good species separate from the rest of the *S. brevicaule* group. SDA of *S. spegazzinii* from the US study (*S. spegazzinii* did not grow well in Peru) labeled as one taxon and the rest of the *S. brevicaule* complex (both north and south members combined) as another taxon identified 15 characters to separate these two groups. Means, ranges, and one standard deviation of the mean for six of the most important characters identified by SDA separating *S. spegazzinii* from the rest of the taxa of the *S. brevicaule*

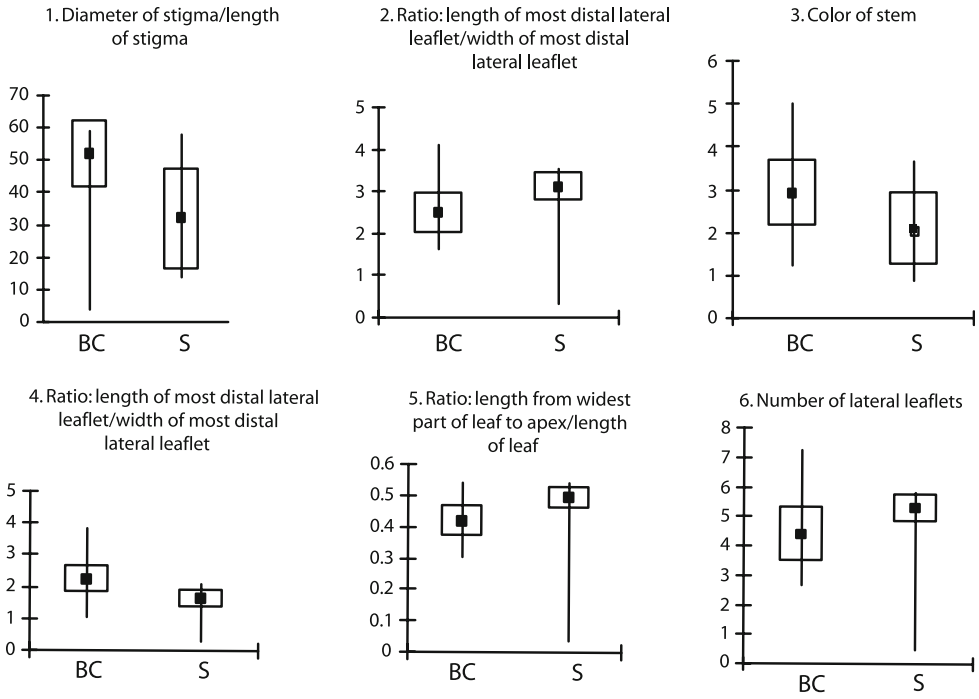

Fig. 5 Mean, ranges, and one standard deviation of the mean for six of the most important characters identified by stepwise discriminate analysis separating *S. spegazzinii* (*S*) from the rest of the taxa of the *S. brevicaule* complex (*BC*) from the study in the US

Table 3 Correlations (comparing similarity values above diagonal) and cophenetic correlations (comparing Neighbor Joining tree topologies below the diagonal) for the various entire data sets of the *S. brevicaule* studies

	MUS[a]	MP[b]	AFLP	RAPD	RFLP
MUS	–	0.29	0.20	0.07	0.09
MP	0.16 (117)	–	0.28	0.10	0.21
AFLP	0.29 (195)	0.22 (144)	–	0.76	0.72
RAPD	0.14 (66)	0.07 (48)	0.74 (76)	–	0.54
RFLP	0.10 (108)	0.13 (76)	0.54 (166)	0.63 (82)	–

The numbers in parentheses are the number of comparisons

[a] Morphological study from the US

[b] Morphological study from Peru

complex in the US study are presented in Fig. 5; all character states overlap tremendously in range.

Concordance of data sets

Correlation coefficients and cophenetic correlation coefficients among both the US and Peruvian morphological data sets and all three molecular marker data sets are presented in Table 3. Similar to the morphological and molecular marker comparisons presented in Miller and Spooner (1999) and Spooner et al. (2005), comparisons among molecular marker results are much higher (0.54–0.76, using the correlation coefficients as representative) than the molecular marker/morphological comparisons (0.20–0.28). Comparison of the morphological/morphological studies (0.29) show intermediate ranges of correlation values.

Discussion

Insights from replicated studies

This is the second replicated morphological study of species boundaries in sect. *Petota*, following a similar one of the wild potato group *Solanum* series *Conicibaccata* Dunal (Fajardo et al. 2008). Both species groups are similar in that they (1) are composed of morphologically similar species, (2) contain disagreement among taxonomists of species boundaries, and (3) contain diploids, tetraploids and hexaploids. The ser. *Conicibaccata* study, like the present study, compared replicated data from the US and Peru, but the US series *Conicibaccata* study was in a greenhouse environment unlike the field environment of the *S. brevicaule* study in the US. Correlation values between ser. *Conicibaccata* morphological replicates were higher (0.62) than US and Peruvian replicates of the *S. brevicaule* complex (0.29) (Table 3). This may be due to the fact that both were in a greenhouse environment, unlike the *S. brevicaule* complex study that compared a field study (US) to a greenhouse study (Peru). However, despite low correlation values, the general conclusions from replicates of both studies were similar in that they defined similar broad groups of species, but showed poor support within the groups.

Comparison of stepwise discriminate analyses

SDA shows the best characters discriminating pre-determined groups. In our study, SDA determined only one of the top five characters to be common among both US and Peruvian studies of the reduced data set of the *S. brevicaule* studies: number of flowers per inflorescence that was ranked first in Peru. The lack of concordance of SDA results could have many causes, but we suggest it is caused by the poor definitions of taxa as coded here, and argues for a reduction of species in the group.

Number of species in the *S. brevicaule* complex

Van den Berg et al. (1998) and Miller and Spooner (1999) came to similar conclusions regarding species in the *S. brevicaule* complex; namely that their most liberal taxonomic interpretation would be to recognize only three wild taxa: (1) the "northern" Peruvian populations (including *S. achacacense* from immediately adjacent Bolivia), (2) the "southern" Bolivian and Argentinean populations, and (3) *S. oplocense*. Despite this greatly reduced number of species, even these three "species" had no species-specific characters and could only be distinguished with great difficulty because there was overlap of the best characters separating them [see Fig. 8 of van den Berg et al. [1998] and Fig. 5 of the present study]. Our Peruvian morphological data support a similar conclusion. While AFLP data (Spooner et al. 2005) support some taxa within the complex (for example the cultivated species as a single species *S. tuberosum*, *S. spegazzinii*, and *S. vernei*) our morphological data show that these will be hard to define morphologically.

Our final taxonomic decisions will use research in progress using DNA sequence data from multiple genes and data from herbarium specimens, including types. DNA sequence data will provide a larger database, to include putatively more discriminatory DNA sequence markers with greater determination of homology, to allow an investigation of possible parents of polyploids relative to extant diploids. However, based on our experience in the field, the results presented here, and supported by an integrative taxonomical approach including molecular data and morphological data from several herbarium specimens and types, we conclude that collapse of many of the species now recognized in the *S. brevicaule* complex, as in many other groups in sect. *Petota*, is unavoidable.

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