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Collapse of species boundaries in the wild potato *Solanum brevicaule* complex (*Solanaceae*, *S.* sect. *Petota*): molecular data

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Key words: *Solanaceae*, sect. *Petota*, *Solanum brevicaule*. – Domestication, hybridization, potatoes, systematics.

Abstract: The *Solanum brevicaule* complex is a group of morphologically very similar wild and cultivated potato taxa (*Solanum* sect. *Petota*). This study uses single to low-copy nuclear RFLPs and RAPDs to investigate their species boundaries and relationships. Cladistic analyses of both data sets are largely concordant with each other and with a recently published phenetic analyses of the same accessions using morphology. All three data sets separate members of the complex into populations from Peru and immediately adjacent northwestern Bolivia, including most cultivated species accessions, and populations from northwestern Bolivia to Argentina. The molecular results suggest that the complex is paraphyletic as currently circumscribed. Many species of the *S. brevicaule* complex should be relegated to synonymy.

According to the latest taxonomic treatment by Hawkes (1990), *Solanum* L. sect. *Petota* Dumort. contains 232 species. They range from the southwestern United States to southern Chile, and from sea level to over 4000 m in elevation. Species concepts in sect. *Petota* have relied mainly on intuitive judgments based on morphology, with additional information provided by ploidy level, geography, crossability relationships, and serology (Hawkes 1990).

The *Solanum brevicaule* Bitter complex (Table 1) is a group of approximately 30 morphologically very similar taxa within sect. *Petota*. This complex was first recognized, in part, by Ugent (1966; first published in Ugent 1970) as a taxonomically confusing group of putative ancestors of the cultivated potato species endemic to central Peru, Bolivia, and northern Argentina. Wild members of the *S. brevicaule* complex are difficult to distinguish from the cultivated members and from each other. No qualitative characters distinguish these species, and they differ only by widely overlapping character states (Spooner & Van Den Berg 1992; Van Den Berg & al. 1996, 1998).

Van Den Berg & al. (1998) examined 256 populations of 30 taxa. They concluded that only three groups could be distinguished with difficulty, and only by a widely overlapping series of morphological character states: (1) Peruvian and

Table 1. Accessions of *Solanum* sect. *Petota* examined. Vouchers are deposited at the herbarium of the National Research Support Program-6, Sturgeon Bay, Wisconsin.

Map location[1]	Study[2]	PI Number[3]	Taxon	Country, Department or Province
1	R, D, M	230512	*S. stenotomum* JUZ. & BUKASOV subsp. *goniocalyx* JUZ. & BUKASOV	Peru, Amazonas
1	R, D, M	230513	*S. stenotomum* subsp. *stenotomum*	Peru, Amazonas
2	R, M	365362	*S. ambosinum* OCHOA	Peru, Ancash
2	R	498266	*S. multiinterruptum* BITTER	Peru, Ancash
3	R, M	498209	*S. ambosinum*	Peru, Ancash
3	R, M	498212	*S. ambosinum*	Peru, Ancash
3	R, M	498213	*S. ambosinum*	Peru, Ancash
3	R	365336	*S. multiinterruptum*	Peru, Ancash
3	R	365338	*S. multiinterruptum*	Peru, Ancash
4	R	442701	*S. blanco-gladosii* OCHOA	Peru, Cajamarca
4	R, D, M	205527	*S. stenotomum* subsp. *stenotomum*	Peru, Huanuco
5	R, M	458402	*S. medians* BITTER	Peru, Lima
6	R	210045	*S. medians*	Peru, Lima
6	R, M	230507	*S. medians*	Peru, Junín
6	R, M	320260	*S. medians*	Peru, Lima
6	R, M	473496	*S. medians*	Peru, Lima
7	R	365337	*S. multiinterruptum*	Peru, Lima
8	R, D	365339	*S. pascoense* OCHOA	Peru, Junín
9	R, D, M	210044	*S. multidissectum* HAWKES	Peru, Junín
10	R, D, M	473492	*S. bukasovii* JUZ.	Peru, Huancavelica
10	R, M	473493	*S. bukasovii*	Peru, Huancavelica
10	R, D[2], M	473494	*S. bukasovii*	Peru, Huancavelica
11	R, M	442697	*S. pampasense* HAWKES	Peru, Apurimac
12	R, D, M	473355	*S. canasense* HAWKES	Peru, Ayacucho
12	R, D, M	210052	*S. multidissectum*	Peru, Ayacucho
12	R, M	275274	*S. pampasense*	Peru, Ayacucho
12	R	275275	*S. pampasense*	Peru, Ayacucho
12	R, M	458381	*S. pampasense*	Peru, Ayacucho
12	R, D, M	195186	*S. stenotomum* subsp. *goniocalyx*	Peru, Ayacucho
13	R, D, M	195188	*S. stenotomum* subsp. *goniocalyx*	Peru, Ayacucho
14	R, D, M	473451	*S. leptophyes* BITTER	Peru, Ayacucho
15	R, D, M	414155	*S. bukasovii*	Peru, Apurimac
16	R, M	458403	*S. abancayense*	Peru, Apurimac
16	R, M	458404	*S. abancayense*	Peru, Apurimac
17	R, D	365353	*S. bukasovii*	Peru, Cuzco

Table 1 (continued)

Map location[1]	Study[2]	PI Number[3]	Taxon	Country, Department or Province
17	R	283084	*S. canasense*	Peru, Puno
17	R, D[2], M	195204	*S. stenotomum* subsp. *stenotomum*	Peru, Cuzco
18	R, D	266385	*S. bukasovii*	Peru, Junin
18	R, D, M	210035	*S. canasense*	Peru, Cuzco
18	R, D, M	246533	*S. canasense*	Peru, Cuzco
18	R, D	265864	*S. canasense*	Peru, Cuzco
18	R	210040	*S. marinasense* VARGAS	Peru, Cuzco
18	R	458380	*S. marinasense*	Peru, Cuzco
18	R	275272	*S. multiinterruptum*	Peru, Cuzco
18	R, D, M	473349	*S. multidissectum*	Peru, Cuzco
18	R, D, M	246536	*S. sparsipilum* JUZ. & BUKASOV	Peru, Cuzco
18	R, D, M	473385	*S. sparsipilum*	Peru, Cuzco
19	R	310944	*S. marinasense*	Peru, Cuzco
19	R	310946	*S. marinasense*	Peru, Puno
20	R, M	473353	*S. multidissectum*	Peru, Cuzco
21	R, M	365314	*S. acroscopicum* OCHOA	Peru, Arequipa
22	R, M	498254	*S. marinasense*	Peru, Arequipa
23	R, D, M	210055	*S. multidissectum*	Peru, Cuzco
23	R, D, M	473352	*S. multidissectum*	Peru, Puno
24	R, D, M	568933	*S. bukasovii*	Peru, Puno
24	R, D, M	568954	*S. bukasovii*	Peru, Puno
25	R, D, M	265863	*S. canasense*	Peru, Puno
25	R, D, M	442696	*S. canasense*	Peru, Puno
26	R, D, M	458378	*S. leptophyes*	Peru, Puno
27	R, M	230495	*S. acroscopicum*	Peru, Tacna
29	R, D, M	558032	*S. achacachense*	Bolivia, La Paz
29	R, D, M	545970	*S. brevicaule*	Bolivia, La Paz
29	R, M	545972	*S. candolleanum* BERTHAULT.	Bolivia, La Paz
30	R, D, M	498284	*S. sparsipilum*	Bolivia, La Paz
31	R, M	473342	*S. leptophyes*	Bolivia, La Paz
32	R, M	265865	*S. canasense*	Bolivia, La Paz
32	R	473360	*S. megistacrolobum* BITTER	Bolivia, La Paz
32	R, D, M	458393	*S. stenotomum* subsp. *goniocalyx*	Bolivia, La Paz
32	R, D, M	280991	*S. tuberosum* L. subsp. *andigena* HAWKES	Bolivia, La Paz
33	R, M	258917	*S. tuberosum* subsp.	Bolivia, Cochabamba

(contd.)

Table 1 (continued)

Map location[1]	Study[2]	PI Number[3]	Taxon	Country, Department or Province
			andigena	
34	R, M	473378	*S. brevicaule* BITTER	Bolivia, Cochabamba
34	R, D[2], M	498111	*S. brevicaule*	Bolivia, Cochabamba
34	R, D, M	498218	*S. brevicaule*	Bolivia, Cochabamba
34	R, M	545971	*S. brevicaule*	Bolivia, Cochabamba
34	R, D, M	473375	*S. sparsipilum*	Bolivia, Cochabamba
34	R, D, M	545980	*S. stenotomum* subsp. *stenotomum*	Bolivia, Cochabamba
34	R	545884	*S. vernei* BITTER & WITTM. subsp. *vernei*	Bolivia, Cochabamba
35	R, M	545879	*S. hondelmannii* HAWKES & HJERT.	Bolivia, Cochabamba
35	R, D, M	498134	*S. sparsipilum*	Bolivia, Cochabamba
36	R, M	498091	*S. avilesii* HAWKES & HJERT.	Bolivia, Santa Cruz
36	R, M	498092	*S. avilesii*	Bolivia, Santa Cruz
36	R, M	498093	*S. avilesii*	Bolivia, Santa Cruz
37	R, D, M	545985	*S. leptophyes*	Bolivia, Oruro
38	R, D, M	258879	*S. tuberosum* subsp. *andigena*	Bolivia, Oruro
39	R, D[4]	545968	*S. brevicaule*	Bolivia, Cochabamba
39	R, D, M	545987	*S. leptophyes*	Bolivia, Potosí
39	R, D, M	545997	*S. leptophyes*	Bolivia, Potosí
39	R	320341	*S.* unidentified	Bolivia, Cochabamba
40	R, D, M	537026	*S. gourlayi* HAWKES subsp. *gourlayi* (2x)	Bolivia, Potosí
40	R, D, M	545865	*S. gourlayi* subsp. *pachytrichum* (HAWKES) HAWKES & HJERT.	Bolivia, Potosí
40	R, M	498071	*S. hondelmannii*	Bolivia, Potosí
40	R	210034	*S. megistacrolobum*	Bolivia, Potosí
40	R, D, M	442693	*S. oplocense* HAWKES (4x)	Bolivia, Potosí
40	R, D, M	498285	*S. sparsipilum*	Bolivia, Potosí
40	R, D, M	442691	*S. sucrense* HAWKES	Bolivia, Potosí
40	R, D[2], M	473392	*S. sucrense*	Bolivia, Potosí
41	R, D, M	458392	*S. oplocense* (4x)	Bolivia, Potosí
41	R, D, M	473506	*S. sucrense*	Bolivia, Potosí
42	R, D, M	545909	*S. oplocense* (6x)	Bolivia, Potosí
42	R, D, M	545888	*S. sucrense*	Bolivia, Potosí
43	R, D, M	545906	*S. oplocense* (6x)	Bolivia, Potosí
44	R, D, M	545975	*S. gourlayi* subsp. *pachytrichum*	Bolivia, Chuquisaca
44	R, D, M	545978	*S. gourlayi* subsp. *pachytrichum*	Bolivia, Chuquisaca
44	R, M	473365	*S. hondelmannii*	Bolivia, Chuquisaca
44	R, M	498067	*S. hondelmannii*	Bolivia, Chuquisaca

Table 1 (continued)

Map location[1]	Study[2]	PI Number[3]	Taxon	Country, Department or Province
44	R, D, M	265882	*S. tuberosum* subsp. *andigena*	Bolivia, Chuquisaca
44	R	310931	*S.* unidentified	Bolivia, Chuquisaca
44	R	545981	*S.* unidentified	Bolivia, Chuquisaca
44	R	545983	*S.* unidentified	Bolivia, Chuquisaca
45	R, D, M	545881	*S. hoopesii* HAWKES & K. A. OKADA	Bolivia, Chuquisaca
45	R, D, M	545882	*S. hoopeseii*	Bolivia, Chuquisaca
45	R	546000	*S. megistacrolobum*	Bolivia, Chuquisaca
45	R, D, M	546030	*S. ugentii* HAWKES & K. A. OKADA	Bolivia, Chuquisaca
45	R, D, M	546032	*S. ugentii*	Bolivia, Chuquisaca
45	R	546003	*S.* unidentified	Bolivia, Chuquisaca
46	R, D, M	498306	*S. sucrense*	Bolivia, Chuquisaca
47	R	500020	*S. chacoense* BITTER	Argentina, Jujuy
47	R, D, M	473182	*S. oplocense* (4x)	Argentina, Jujuy
47	R, D, M	473185	*S. oplocense* (6x)	Argentina, Jujuy
47	R, D, M	473251	*S. tuberosum* subsp. *andigena*	Argentina, Jujuy
48	R, D, M	210038	*S. gourlayi* subsp. *gourlayi* (4x)	Argentina, Jujuy
48	R, D, M	558107	*S. oplocense* (4x)	Argentina, Jujuy
48	R, M	558108	*S. oplocense* (6x)	Argentina, Jujuy
49	R, D	473019	*S. gourlayi* subsp. *gourlayi* (2x)	Argentina, Jujuy
49	R, D, M	500022	*S. gourlayi* subsp. *gourlayi* (2x)	Argentina, Jujuy
49	R, D, M	472918	*S. gourlayi* subsp. *gourlayi* (4x)	Argentina, Jujuy
49	R, D, M	473026	*S. gourlayi* subsp. *gourlayi* (4x)	Argentina, Jujuy
49	R, D	498315	*S. gourlayi* subsp. *gourlayi* (4x)	Argentina, Jujuy
49	R, D, M	472991	*S. gourlay* subsp. *vidaurrei* HAWKES & HJERT	Argentina, Jujuy
49	R, D, M	473004	*S. gourlayi* subsp. *vidaurrei*	Argentina, Jujuy
49	R, M	320333	*S. vernei* BITTER & WITTM. subsp. *ballsii* (HAWKES) HAWKES & HJERT.	Argentina, Jujuy
49	R, M	473303	*S. vernei* subsp. *ballsii*	Argentina, Jujuy
49	R, M	558150	*S. vernei* subsp. *vernei*	Argentina, Jujuy
50	R, D, M	558067	*S. gourlayi* subsp. *gourlayi* (2x)	Argentina, Jujuy

(contd.)

Table 1 (continued)

Map location[1]	Study[2]	PI Number[3]	Taxon	Country, Department or Province
51	R, D, M	472911	*S. gourlayi* subsp. *vidaurrei*	Argentina, Salta
51	R, D	473106	*S. gourlayi* subsp. *vidaurrei*	Argentina, Salta
52	R, D, M	472995	*S. gourlayi* subsp. *vidaurrei*	Argentina, Salta
53	R, M	458370	*S. vernei* subsp. *ballsii*	Argentina, Salta
53	R, M	500070	*S. vernei* subsp. *ballsii*	Argentina, Salta
53	R, M	473309	*S. vernei* subsp. *vernei*	Argentina, Salta
54	R, D, M	473095	*S. gourlayi* subsp. *gourlayi* (2x)	Argentina, Salta
54	R	473158	*S. megistacrolobum*	Argentina, Salta
55	R, D, M	473077	*S. gourlayi* subsp. *gourlayi* (2x)	Argentina, Salta
55	R, M	473060	*S. incamayoense* K. A. OKADA & A. M. CLAUSEN	Argentina, Salta
55	R, M	473067	*S. incamayoense*	Argentina, Salta
55	R, M	473069	*S. incamayoense*	Argentina, Salta
55	R	473070	*S. incamayoense*	Argentina, Salta
55	R, M	500048	*S. incamayoense*	Argentina, Salta
55	R	458335	*S. spegazzinii* BITTER	Argentina, Salta
55	R, D, M	473269	*S. tuberosum* subsp. *andigena*	Argentina, Salta
56	R	472816	*S. chacoense*	Argentina, Salta
56	R, D, M	558062	*S. gourlayi* subsp. *gourlayi* (4x)	Argentina, Salta
56	R	500029	*S. megistacrolobum*	Argentina, Salta
57	R	275138	*S. chacoense*	Argentina, Tucumán
57	R, M	472988	*S. spegazzinii*	Argentina, Tucumán
58	R	472936	*S. kurtzianum* BITTER & WITTM.	Argentina, Catamarca
58	R	472952	*S. kurtzianum*	Argentina, Catamarca
58	R, M	320299	*S. spegazzinii*	Argentina, Catamarca
58	R, M	320332	*S. vernei* subsp. *vernei*	Argentina, Catamarca
59	R	472830	*S. chacoense*	Argentina, La Rioja
59	R	472924	*S. kurtzianum*	Argentina, La Rioja
59	R	472948	*S. kurtzianum*	Argentina, La Rioja
59	R	558208	*S. kurtzianum*	Argentina, La Rioja
59	R, M	458337	*S. spegazzinii*	Argentina, La Rioja
59	R, M	472966	*S. spegazzinii*	Argentina, La Rioja
59	R, M	472990	*S. spegazzinii*	Argentina, La Rioja
n	R	365317	*S. ambosinum*	Peru, Pasco
n	R, M	247360	*S. andreanum* BAKER	Columbia, Nariño

Table 1 (continued)

Map location[1]	Study[2]	PI Number[3]	Taxon	Country, Department or Province
n	R	320345	*S. andreanum*	Columbia, Cauca
n	R	561648	*S. andreanum*	Ecuador, Napo
n	R, M	561658	*S. andreanum*	Ecuador, Bolívar
n	R, M	320265	*S. brachistotrichum* (BITTER) RYDB.	Mexico, Chihuahua
n	R, M	558460	*S. brachistotrichum*	Mexico, Jalisco
n	R	320294	*S. chacoense*	Argentina, Buenos Aires
n	R, M	186181	*S. curtilobum* JUZ. & BUKASOV	Peru
n	R, M	225649	*S. curtilobum*	Columbia
n	R, M	258900	*S. curtilobum*	Bolivia
n	R, M	275156	*S. fendleri* A. GRAY	USA, New Mexico
n	R, M	458413	*S. fendleri*	Mexico, Chihuahua
n	R, M	458420	*S. fendleri*	USA, Arizona
n	R, M	498238	*S. fendleri*	Mexico, Chihuahua
n	R, M	558395	*S. fendleri*	Mexico, Baja California
n	R	558185	*S. kurtzianum*	Argentina, Mendoza
n	R	473468	*S. limbaniense* OCHOA	Peru
n	R, D, M	234011	*S. stenotomum* subsp. *stenotomum*	Bolivia
n	R, D, M	225628	*S. tuberosum* subsp. *andigena*	Columbia, Santander
n	R, D, M	281208	*S. tuberosum* subsp.	Peru
n	R, D, M	161401	*S. tuberosum* subsp. *tuberosum*	Mexico, Federal Distrito
n	R	245940	*S. tuberosum* subsp. *tuberosum*	Chile, Tarapacá
n	R, M	275256	*S. verrucosum* SCHLTDL.	Mexico, Michoacán
n	R, M	275257	*S. verrucosum*	Mexico, Michoacán
n	R, M	498061	*S. verrucosum*	Mexico, Coahuila
n	R, M	545745	*S. verrucosum*	Mexico, Nuevo León
n	R, M	545747	*S. verrucosum*	Mexico, Mexico
n	R, M	558482	*S. verrucosum*	Mexico, Mexico
n	R	234009	*S.* unidentified	Bolivia

[1]Generalized map areas of members of the *Solanum brevicaule* complex (Fig. 1; *n* taxa outside the area of the complex not mapped. These areas correspond to those in VAN DEN BERG & al. (1998); all but area 28 have representatives in this study. [2]*R* accession used in the RFLP study, *D* accession used in the RAPD study, superscript indicates the number of times sampled, *M* accession used in the morphological study. [3]United states Department of Agriculture Plant Introduction Numbers

immediately adjacent northwestern Bolivian accession (including wild species and all the cultigens), (2) northwestern Bolivian and Argentinean accessions and *S. verrucosum* from Mexico (including only wild species), and (3) the Bolivian and Argentinean wild species *S. oplocense*. In practicable taxonomic application (i.e. without resorting to multivariate analyses), however, only *S. oplocense* can be distinguished with difficulty from these two geographic groups, and the geographic groups cannot be distinguished from each other. The morphological data (VAN DEN BERG & al. 1998) suggested that many of these species should be relegated to synonymy.

Most of the taxa are widely interfertile, at least in early generations (HAWKES 1958; HAWKES & HJERTING 1969, 1989; OCHOA 1990). Most taxa are diploid and interbreed where they overlap (HAWKES 1990). This suggests that the species as currently recognized have recently evolved from a common ancestor, form a single gene pool, or are perhaps even conspecific.

This molecular study uses a subset of the accessions in the morphological study of VAN DEN BERG & al. (1998) but adds additional putative outgroup taxa (*S. andreanum*, *S. blanco-galdosii*, *S. chacoense*, *S. kurtzianum*, *S. limbaniense*, *S. megistacrolobum*, *S. multiinterruptum*, and *S. pascoense*). Two molecular markers, single-to-low copy nuclear Restriction Fragment Length Polymorphisms (RFLPs) and Random Amplified Polymorphic DNA (RAPDs) were used. This study investigates concordance among morphological and molecular data sets to explore species boundaries and relationships of the *S. brevicaule* complex.

Materials and methods

Species in RFLP analysis. A total of 196 accessions were examined: 154 accessions of 31 taxa of the *S. brevicaule* complex, and 42 accessions of 14 putative outgroups. Of the 196 accessions, 145 were identical to the morphological analysis of VAN DEN BERG & al. (1998 Table 1). The putative outgroups are: *S. limbaniense* (ser. *Conicibaccata* BITTER), *S. fendleri* (ser. *Longipedicellata* BUKASOV), *S. megistacrolobum* (ser. *Megistacroloba* CÁRDENAS & HAWKES), *S. brachistotrichum* (series *Pinnatisecta* [RYDB.] HAWKES), *S. blanco-galdosii* and *S. pascoense* (ser. *Piruana* HAWKES), *S. andreanum*, *S. acroscopicum*, *S. kurtzianum*, *S. multiinterruptum*, *S. vernei* subsp. *ballsii*, subsp. vernei, and *S. verrucosum* (ser. *Tuberosa* [RYDB.] HAWKES), and *S. chacoense* (ser. *Yungasensia* CORRELL). We chose *S. brachistotrichum* as a representative of a primitive member of sect. *Petota*, based on hypotheses of HAWKES (1990) and chloroplast DNA data (SPOONER & SYTSMA 1992, SPOONER & CASTILLO 1997). We chose *S. fendleri* and *S. verrucosum* because they appeared to be possible morphological candidates for inclusion into the complex (VAN DEN BERG & al. 1998), and because *S. verrucosum* is the northern-most representative of ser. *Tuberosa*. The rest of the taxa represent morphologically distinctive species chosen as other representatives of ser. *Tuberosa* (to which the *S. brevicaule* complex belongs), and other series in sect. *Petota*. We chose the accessions to represent a geographically well dispersed sample, and they occur in 58 of the 59 generalized map areas in VAN DEN BERG & al. (1998; Fig. 1). Six unidentified accessions from Bolivia and Peru were included. All accessions came from the National Research Support Program-6 (NSRP-6; BAMBERG & al. 1996). Identifications of the species were provided by visiting taxonomists during visits to NRSP-6 to inspect living representatives in field plots (HANNEMAN 1989).

RFLP probes. The RFLP analysis used 29 unmapped probes from a random genomic library of the wild potato *S. phureja* JUZ. & BUKASOV (HOSAKA & SPOONER 1992; P10, P93,

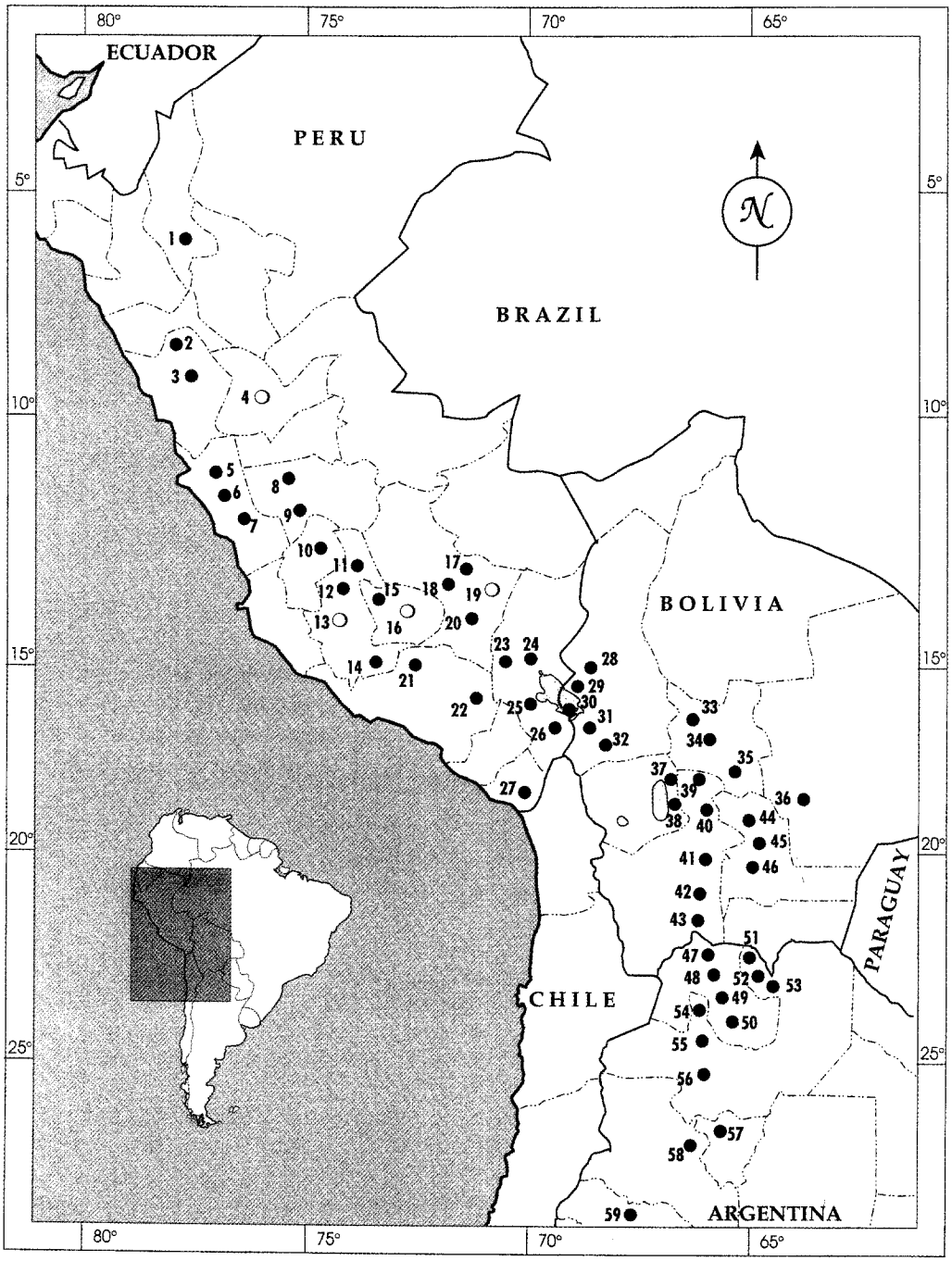


Fig. 1. Map showing the 59 generalized areas of accessions of the *S. brevicaule* complex (see Table 1). Map areas are identical to a companion morphological study in Van Den Berg & al. (1998); map area 28 is not sampled in this molecular study), and correspond to those in Table 1. Solid circles are areas with good locality data; open circles are areas that can be mapped only to Department (or Province)

P101, P122, P140, P209, P212, P215, P247, P256, P265, P278, P279, P292, P307, P330, P332, P368, P374, P380, P392, P403, P417, P463, P473, P543, P573, P620, P747) and 24 tomato probes mapped to 20 of the 24 potato chromosome arms (TANKSLEY & al. 1992; three cDNA clones, CD2, CD14, CD67; and 21 genomic clones, TG16, TG18, TG20, TG23, TG24, TG31, TG36, TG48, TG61, TG63, TG65, TG57, TG71, TG94, TG122, TG130, TG134, TG141, TG152, TG180, TG275). The probes were amplified by the Polymerase Chain Reaction and radiolabeled with ^{32}P-dCTP by the method of FEINBERG & VOLGELSTEIN (1984).

RFLPs, DNA isolation and restriction site comparison. Five g of leaf tissue from a bulk of ten plants were used for DNA extraction. Bulk extractions sample more diversity within a population, especially in predominantly outcrossing taxa like most members of the complex, however single plant studies allow within and among population diversity estimates (ALDRICH & DOEBLEY 1992, BRUBAKER & WENDEL 1994). Because the goal of the present study is to investigate species boundaries within the *S. brevicaule* complex, we designed the study to sample as many alleles as possible within populations and to examine more populations rather than more individuals within populations. This method may overestimate similarity between populations since rare alleles are scored equal to common alleles and variation within populations is not addressed (SONG & al. 1990).

All isolation and purification protocols followed GIANNATTASIO & SPOONER (1994) except that 6×CTAB was substituted for 2×CTAB. Five µg of each DNA sample were separately digested with *Dra*I and *Eco*RI restriction endonucleases according to manufacturer's instructions. These enzymes are effective in revealing polymorphism in sect. *Petota* (GIANNATTASIO & SPOONER 1994, MILLER & SPOONER 1996). Gel electrophoresis, Southern transfers, hybridization, and autoradiography followed GIANNATTASIO & SPOONER (1994). Polymorphic bands were converted to one (presence) and zero (absence) data. Bands were scored from only one enzyme per probe to avoid the possibility of scoring redundant data (GIANNATTASIO & SPOONER 1994).

RAPDs, species. The goal of the RAPD study was to determine if these markers could distinguish species and reveal relationships within the complex similar to or better than the RFLP data. The RAPD study used a subset of 88 accessions from the RFLP study (Table 1), including one outgroup accession of *S. pascoense*. The same DNA samples were used for the RFLP and RAPD analyses.

RAPD primers. Thirty-six 10-mer primers (A2, A4, A12, A16, B14, D2, E3, F5, Q1, R8, R9, R12, R13, S3, S7, S9, S11, S17, S19, T8, W2, W3, AA2, AA3, AA10, AA12, AA14, AA17, AB4, AG2, AN15, AR12, AN16, AN18, AO1, AR16; Operon Technologies, Alameda, California) were used to amplify DNA. These primers are useful in potato systematic studies (SPOONER & al. 1996, MILLER & SPOONER 1996, SPOONER & al. 1997).

RAPD amplification and band scoring. RAPD reactions were performed in 10 µl volumes in a Perkin Elmer 9600 Thermal Cycler™ programmed for 45 cycles following the cycling protocol of SKROCH & NIENHIUS (1995). Polymorphic bands were converted to one (presence) and zero (absence) data. Five populations were amplified and resolved by electrophoresis multiple times to help score comigrating bands (Table 1). A control reaction containing only water was included and bands found in this lane were not scored in any lane.

Parsimony analyses. We chose to analyze our RFLP and RAPD data with parsimony analysis as a method to search for clades that are stable across data sets. This may be a way to explore the possible reticulation/divergence boundary, where species may be defined (below). Wagner parsimony analyses were performed with PAUP 3. 1. 1 (SWOFFORD 1993). A four-step search strategy suggested by OLMSTEAD & PALMER (1994) was performed with all data sets: 1) 10000 random replicates were performed with nearest neighbor interchange (NNI) branch swapping algorithm; 2) the shortest trees from step one were used as

starting trees with tree bisection and reconnection (TBR) option; 3) the shortest trees from step two were used as starting trees, for a search using NNI for multiple parsimonious trees (MULPARS); 4) trees from step three were used as starting trees for a search using TBR and MULPARS with 10000 as upper limit of the trees saved, due to computer memory restriction. Strict consensus trees were computed from all of the equally most parsimonious trees. To search for possible shorter trees, an inverse constraint search was performed on the strict consensus trees, using 1000 replicates, following the strategy outlined in RICE & al. (1997; random order searches using TBR and no MULPARS, retaining two trees greater or equal to a tree of length less than the consensus tree).

Considering hypotheses of HAWKES (1990) and chloroplast DNA data (SPOONER & SYTSMA 1992, SPOONER & CASTILLO 1997) the entire RFLP data set was rooted on *S. brachistotrichum*. Considering the results of the RFLP analysis, *S. pascoense* was used as the outgroup for all RAPD analyses. Consistency indices (CI) and retention indices (RI) are given.

Topology-based comparisons of RFLP and RAPD data sets. Data subset pairs were generated between RFLP and RAPD data sets (containing 88 accessions in common) and compared by incongruity indices. The MICKEVICH & FARRIS (1981) incongruity index (I_{MF}) measures incongruence by comparing the number of extra steps needed in an individual data set to the number of extra steps needed in a combined data set tree. SWOFFORD (1991) suggested that the I_{MF} may underestimate incongruence and proposed an alternative (I_M) based on constraining the topology of one data set on the data of the other.

Distance-based comparisons of morphology, RFLP, and RAPD data sets. Data subset pairs were compared between morphology (VAN DEN BERG & al. 1998), RFLP, and RAPD data sets (145 accessions in common between the morphology and RFLP; 88 between RFLP and RAPD; 79 between the morphology and RAPD) by comparing their dissimilarity matrices by Spearman-rank correlation (SAS 1995). This non-parametric statistic compares absolute ranks of individual differences among populations and is a method to compare the morphological phenetic results (VAN DEN BERG & al. 1998) to our molecular data. Data subsets also were compared from 20 randomly selected potato RFLP probes and 20 tomato RFLP probes mapped to 20 of the 24 potato chromosome arms (TANKSLEY & al. 1992). Similarity matrices for the morphological data used a distance matrix (VAN DEN BERG & al. 1998), the RFLP data used a simple matching coefficient, and the RAPD data used a Jaccard's coefficient, because of the typically dominant nature of RAPD markers and reduced chance that 0/0 matches represent homology. The complement (1-x) of the molecular similarity matrix was used for comparison with distance matrix of by VAN DEN BERG & al. (1998).

Testing hybridization hypotheses. Some taxa within the *S. brevicaule* complex are putative hybrids. HAWKES (1958) and CRIBB & HAWKES (1986) hypothesized the hybrid origin of *S. tuberosum* subsp. *andigena* from *S. stenotomum* and *S. sparsipilum*; BRUCHER (1964) from *S. vernei* and *S. stenotomum*; and MATSUBAYASHI (1981) from *S. phureja* and *S. stenotomum*. *Solanum phureja* is not included in this study, so this latter hypothesis cannot be addressed. *Solanum sucrense* is a hypothesized hybrid between *S. oplocense* and *S. tuberosum* subsp. *andigena* (ASTLEY & HAWKES 1979). These hypotheses were tested by examining whether the proposed parental species have species-specific bands and, if so, by searching for possible additive banding profiles in the putative hybrids.

Results

RFLP polymorphisms. The 53 probes generated 238 polymorphic bands for the 196 accessions of 45 taxa (data listed in MILLER 1997). Only 303 of 46648 data

points (0.65%) were scored as missing because of apparently poor restriction digests. Two hundred eighteen bands (92%) are informative in the parsimony analyses. Diploid accessions averaged 1.2 polymorphic bands per probe/enzyme combination while tetraploids average 1.4 bands, with significantly more bands in the tetraploids (p from t test < 0.0001). However within *S. gourlayi* (2x, 1.3 bands and 4x, 1.4 bands) and *S. oplocense* (4x, 1.4 bands and 6x, 1.3 bands) there are no significant differences in the mean band number between ploidy levels. This suggests that these populations are of autopolyploid origin.

To determine whether the bulk DNA samples of ten plants was sampling more alleles than single plants, bulk sample RFLP banding patterns were compared to identical probe/enzyme combinations of MILLER & SPOONER (1996) that analyzed three separate individuals per accession. A general survey indicates that data from the three individual plants of that study are combined in the bulk samples of the present study. For example, with probe/enzyme combination 209/*Dra*I two individual plants of *S. spegazzinii* 320299 have a single band while the third plant had a second band. Both bands are found in the bulk extraction. Probe/enzyme 265/*Eco*RI show a simple two banded pattern for all three plants of *S. hondelmannii* accession 473365, however the bulk extraction also shows an additional band that is found in individual plants of another *S. hondelmannii* accession.

RFLP parsimony (Figs. 2, 3). The first four steps of parsimony analyses found at least 10000 equally parsimonious trees of 2286 steps. The subsequent constraint analysis found a 2280 step tree; TBR and MULPARS using this as a starting tree produced at least 10000 equally parsimonious trees of 2280 steps with CI = 0.10 and RI = 0.64 (Fig. 2). The strict consensus tree (Fig. 3) defines: 1) a monophyletic clade B containing species from northwestern Bolivia to northern Argentina, and including the previously presumed outgroup taxa *S. chacoense*, *S. fendleri*, *S. kurtzianum*, *S. megistacrolobum*, *S. vernei*, and *S. verrucosum*; and 2) a basal grade A containing species from Peru and extreme northwestern Bolivia, and containing 21 of the 22 cultigens (all except one of the three accessions of *S. curtilobum*, PI 258900).

Clade B includes one of the four accessions of *S. andreanum*, *S. avilesii*, *S. brevicaule*, *S. chacoense*, *S. fendleri*, *S. gourlayi* subsp. *gourlayi* 2x, 4x, subsp. *pachytrichum* and subsp. *vidaurrei*, *S. hondelmannii*, *S. hoopesii*, *S. incamayoense*, *S. kurtzianum*, *S. leptophyes*, *S. megistacrolobum*, three of the six accessions of *S. multidissectum*, *S. oplocense* 4x, *S. oplocense* 6x, *S. pampasense*, *S. sparsipilum*, *S. spegazzinii*, *S. sucrense*, *S. ugentii*, *S. vernei* subsp. *ballsii* and *vernei*, and *S. verrucosum*. *Solanum fendleri*, *S. hoopesii*, *S. kurtzianum*, and *S. pampasense* form taxon-specific clades. The Mexican species *S. fendleri* and *S. verrucosum* form a clade, except for the anomalous placement of one of the four accessions of *S. andreanum*.

Grade A includes: *S. abancayense*, *S. achacachense*, *S. ambosinum*, *S. bukasovii*, *S. canasense*, *S. candolleanum*, *S. limbaniense*, *S. marinasense*, the other three of the six accessions of *S. multidissectum*, one of the five accessions of *S. multiinterruptum*, and 21 of the 22 cultivated accessions examined (*S. curtilobum*, *S. stenotomum* subsp. *stenotomum* and subsp. *goniocalyx*, *S. tuberosum* subsp. *andigena* and subsp. *tuberosum*). These cultivated taxa do not follow the

geographic separation of the wild taxa but rather are more widely distributed from Mexico to Chile (Table 1). There are no taxon-specific clades. Clade B and grade A generally agree with the morphological groups of VAN DEN BERG & al. (1998), except that *S. achacachense* and all accessions of *S. multidissectum* group with species from grade A (from Peru), and *S. vernei* subspp. *ballsii* and *vernei* group with species from clade B.

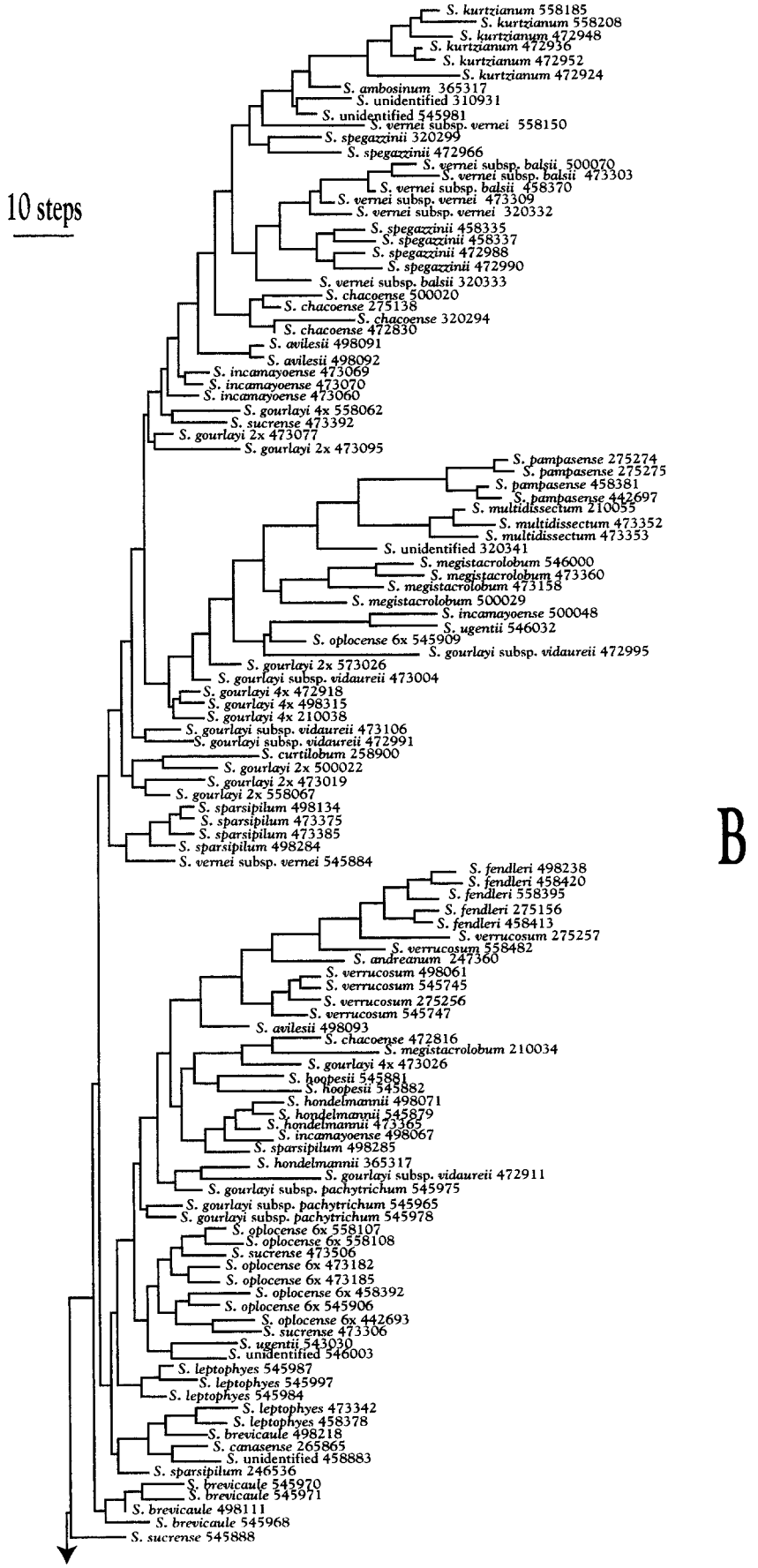
Clade B and grade A are very weakly separated (by a single homoplasious character), similar to poor separation of these two groups with the morphological data (VAN DEN BERG & al. 1998). Two "misplaced" accessions fall outside the geographical area of others of those species. *Solanum canasense* 265865 is from Bolivia while the rest are from Peru. *Solanum leptophyes* 473451 is from Peru while the rest are from Bolivia.

Most putative outgroup taxa are basal to both clades A and B. The outgroups *S. acroscopicum*, *S. brachistotrichum*, and *S. medians* are the only taxa specific outgroups identified. To see if the sole use of *S. pascoense* as an outgroup in the RAPD analysis corresponded to the results of the RFLP analysis, we analyzed the RFLP data with this species as the sole outgroup. The two clades remained the same except that *S. kurtzianum*, *S. megistacrolobum*, *S. multidissectum* (three of six accessions), *S. pampasense*, and two *S. gourlayi* subsp. *vidaurrei* accessions became basal to the entire *S. brevicaule* complex instead of part of grade B (MILLER 1997). It is possible that RFLP probes exhibit more homoplasy with increasing taxonomic distance, and it is more appropriate to use a closer outgroup (*S. pascoense* instead of *S. brachistotrichum*). The only non *S. brevicaule* complex taxa now embedded within the complex are *S. chacoense*, *S. vernei* and the Mexican species *S. fendleri* and *S. verrucosum*.

RAPD polymorphisms. The 36 RAPD primers generated 167 polymorphic bands for the 88 populations of 20 taxa (data listed in MILLER 1997). Only 68 of 15865 data points (0.4%) were scored as missing because of apparently poor amplification. One hundred fifty-five bands (65%) are informative in the parsimony analyses. Diploid accessions average 1.9 bands per primer while tetraploids average 2.2 bands per primer ($p < 0.0001$). Within *S. gourlayi* (2x, 1.9 bands and 4x, 2.0 bands) and *S. oplocense* (4x, 2.1 bands and 6x, 2.0 bands), there are no significant differences in the mean band number between ploidy levels, as in the RFLP results.

RAPD parsimony. The accessions used in the RAPD analysis are a subset of the RFLP analysis, and with only *S. pascoense* as the outgroup. The RAPD data accessions run multiple times (Table 1) grouped together before grouping with different accessions. The first four steps of parsimony analysis of the entire RAPD data set yielded 28 most parsimonious trees of 1485 steps ($CI = 0.11$; $RI = 0.55$); no shorter trees were found with a constraint analysis. The strict consensus tree (Fig. 4), like the strict consensus RFLP tree, delineates a monophyletic clade B and a basal grade A.

Topology-based comparisons of RFLP and RAPD data sets. The incongruity between the RFLP and RAPD data sets, as measured by the incongruity indices I_M and I_{MF} were 17.5% and 5.9% respectively. As expected, the I_M is higher than the I_{MF} (SWOFFORD 1991). The total evidence analysis of the 88 accessions in common used *S. pascoense* as outgroup. The first four steps of


10 steps
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S. kurtzianum 558208
S. kurtzianum 472948
S. kurtzianum 472936
S. kurtzianum 472952
S. kurtzianum 472924
S. ambosinum 365317
S. unidentified 310931
S. unidentified 545981
S. vernei subsp. vernei 558150
S. spegazzinii 320299
S. spegazzinii 472966
S. vernei subsp. balsii 500070
S. vernei subsp. balsii 473303
S. vernei subsp. balsii 458370
S. vernei subsp. vernei 473309
S. vernei subsp. vernei 320332
S. spegazzinii 458335
S. spegazzinii 458337
S. spegazzinii 472988
S. spegazzinii 472990
S. vernei subsp. balsii 320333
S. chacoense 500020
S. chacoense 275138
S. chacoense 320294
S. chacoense 472830
S. avilesii 498091
S. avilesii 498092
S. incamayoense 473069
S. incamayoense 473070
S. incamayoense 473060
S. gourlayi 4x 558062
S. sucrense 473392
S. gourlayi 2x 473077
S. gourlayi 2x 473095
S. pampasense 275274
S. pampasense 275275
S. pampasense 458381
S. pampasense 442697
S. multidissectum 210055
S. multidissectum 473352
S. multidissectum 473353
S. unidentified 320341
S. megistacrolobum 546000
S. megistacrolobum 473360
S. megistacrolobum 473158
S. megistacrolobum 500029
S. incamayoense 500048
S. ugentii 546032
S. oplocense 6x 545909
S. gourlayi subsp. vidaureii 472995
S. gourlayi 2x 573026
S. gourlayi subsp. vidaureii 473004
S. gourlayi 4x 472918
S. gourlayi 4x 498315
S. gourlayi 4x 210038
S. gourlayi subsp. vidaureii 473106
S. gourlayi subsp. vidaureii 472991
S. curtilobum 258900
S. gourlayi 2x 500022
S. gourlayi 2x 473019
S. gourlayi 2x 558067
S. sparsipilum 498134
S. sparsipilum 473375
S. sparsipilum 473385
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S. fendleri 498238
S. fendleri 458420
S. fendleri 558395
S. fendleri 275156
S. fendleri 458413
S. verrucosum 275257
S. verrucosum 558482
S. andreanum 247360
S. verrucosum 498061
S. verrucosum 545745
S. verrucosum 275256
S. verrucosum 545747
S. avilesii 498093
S. chacoense 472816
S. megistacrolobum 210034
S. gourlayi 4x 473026
S. hoopesii 545881
S. hoopesii 545882
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B

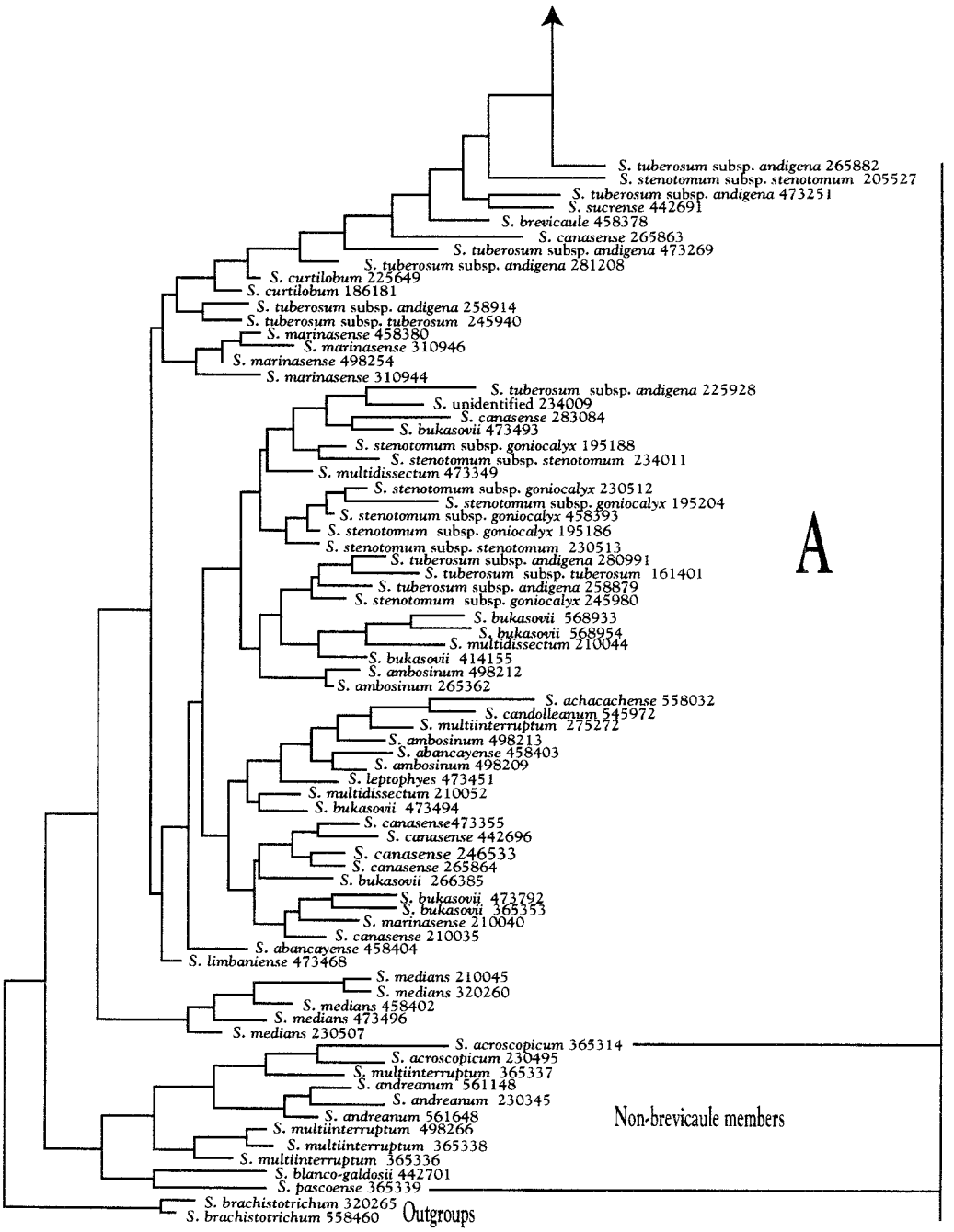
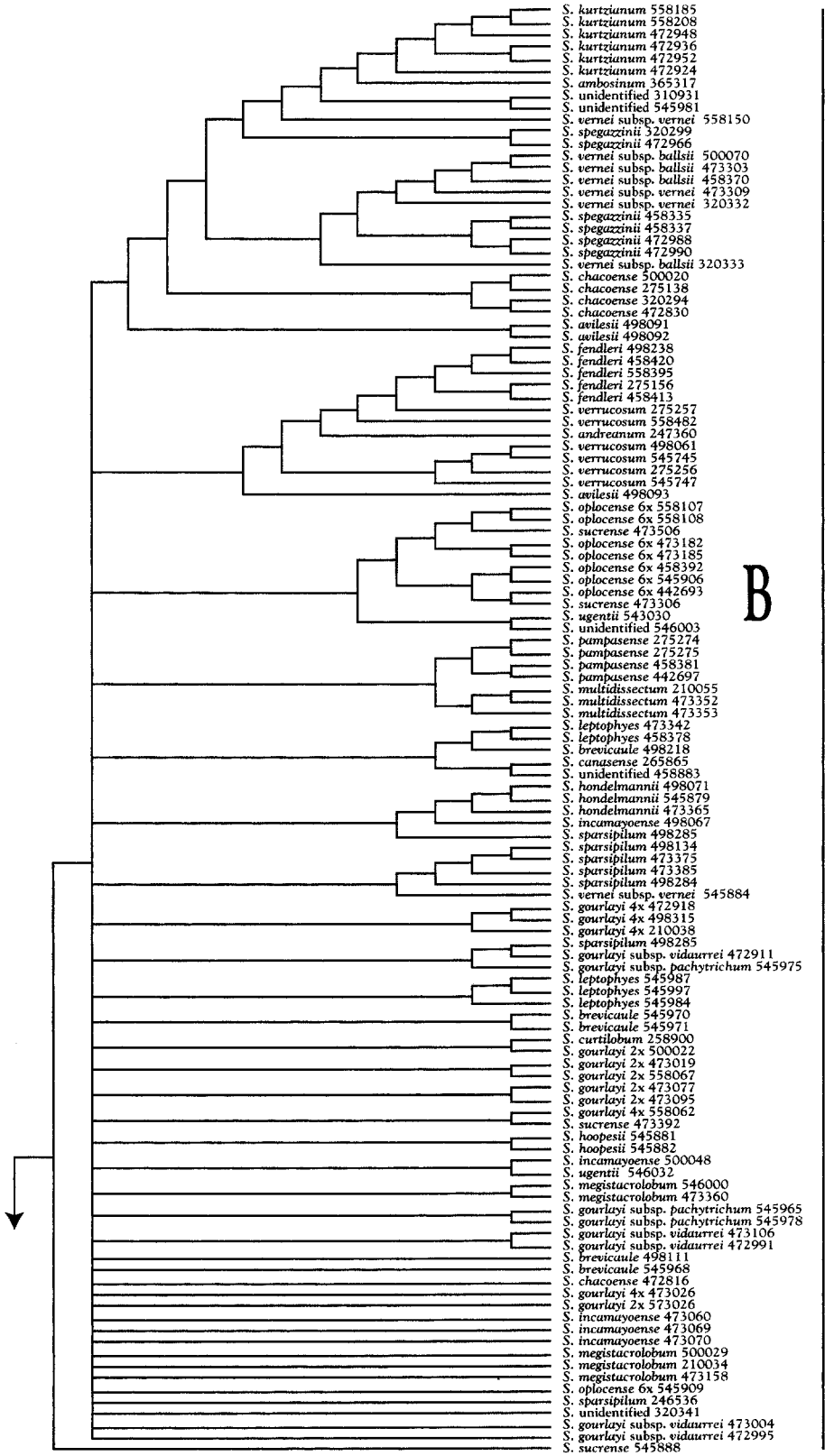


Fig. 2. One of 10000 equally most parsimonious trees of 2280 steps based on the entire RFLP data set. Vertical bars indicate groups that correspond to groups in Figs. 2–5

parsimony analysis of the entire RAPD data set yielded 88 most parsimonious trees of 2578 steps (CI = 0.12; RI = 0.55); no shorter trees were found with a constraint analysis. The strict consensus tree, like both the RFLP and RAPD trees, delineates a monophyletic clade B and a basal grade A, like in the RFLP data (Fig. 5).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S. kurtzianum 558208
S. kurtzianum 472948
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S. kurtzianum 472952
S. kurtzianum 472924
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S. spegazzinii 320299
S. spegazzinii 472966
S. vernei subsp. ballsii 500070
S. vernei subsp. ballsii 473303
S. vernei subsp. ballsii 458370
S. vernei subsp. vernei 473309
S. vernei subsp. vernei 320332
S. spegazzinii 458335
S. spegazzinii 458337
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S. spegazzinii 472990
S. vernei subsp. ballsii 320333
S. chacoense 500020
S. chacoense 275138
S. chacoense 320294
S. chacoense 472830
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S. gourlayi 2x 473077
S. gourlayi 2x 473095
S. gourlayi 4x 558062
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S. megistacrolobum 473360
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S. gourlayi subsp. viduurrei 472991
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S. gourlayi 4x 473026
S. gourlayi 2x 573026
S. incamayoense 473060
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S. incamayoense 473070
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S. megistacrolobum 210034
S. megistacrolobum 473158
S. oplocense 6x 545909
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S. sucrense 545888
B

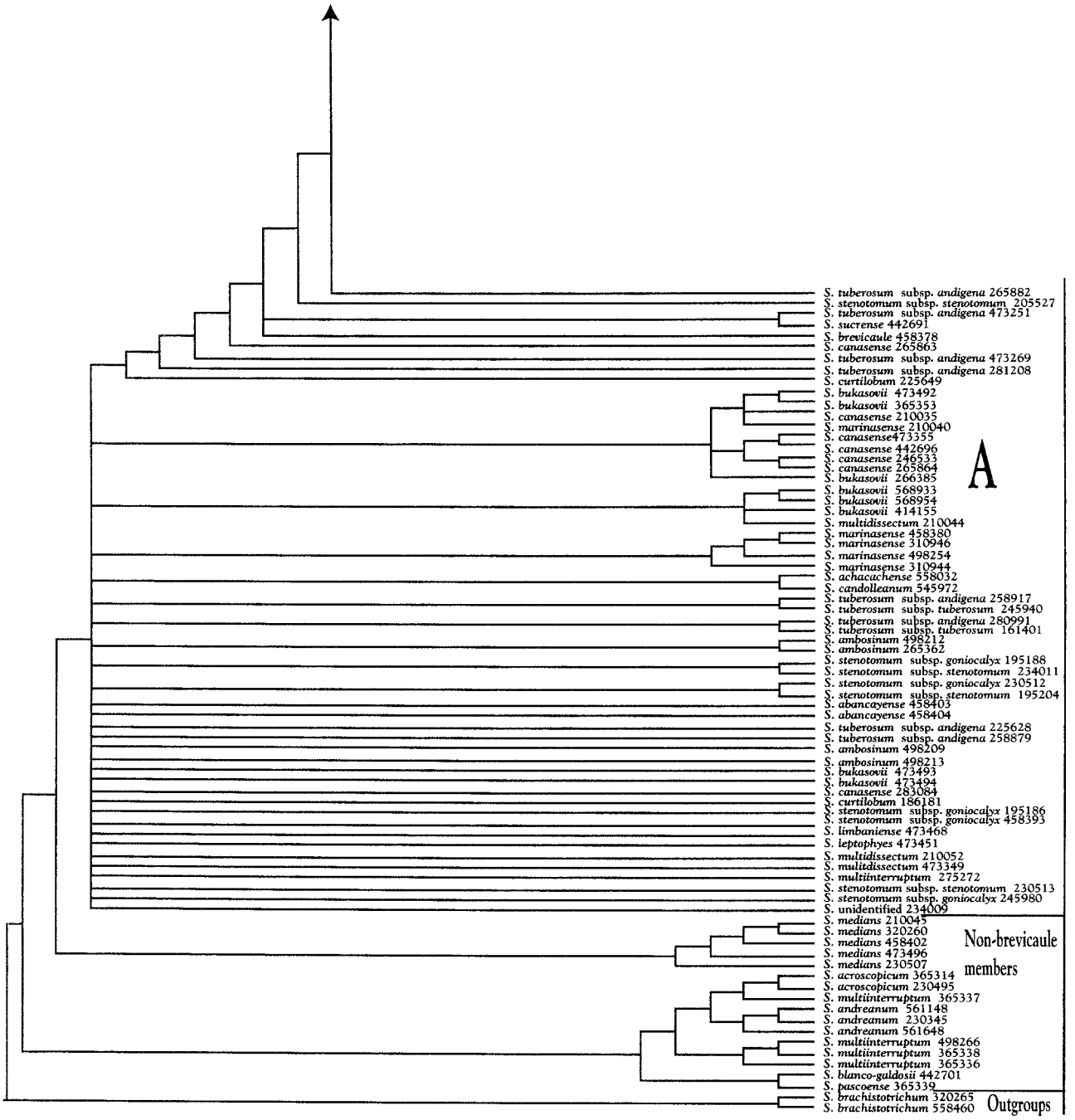

Fig. 3. Strict consensus parsimony cladogram of 10000 equally most parsimonious trees of 2280 steps based on the entire RFLP data set. Vertical bars indicate groups that correspond to groups in Figs. 2, 4 and 5

Distance-based comparisons of morphology, RFLP and RAPD data sets. Spearman-rank correlation between RFLP and RAPD ($r = 0.76$) was higher than either RFLP or RAPD data sets were to the morphology ($r = 0.24$ and 0.25). The mapped tomato RFLP probes resulted in 4.6 bands per probe while the unmapped

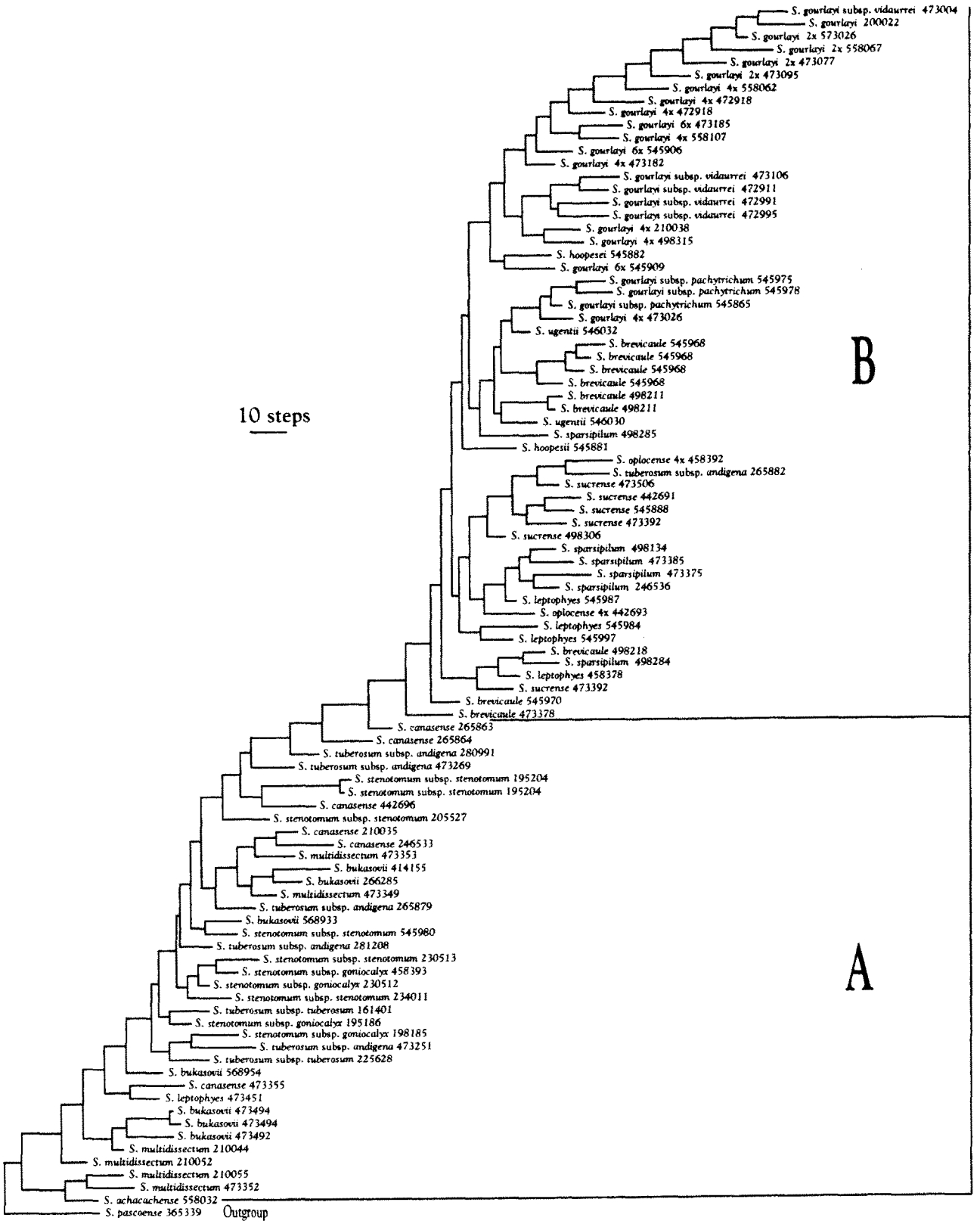

Fig. 4. One of 28 equally most parsimonious trees 1485 steps based on the entire RAPD data set. Vertical bars indicate groups that correspond to groups in Figs. 2, 3 and 5

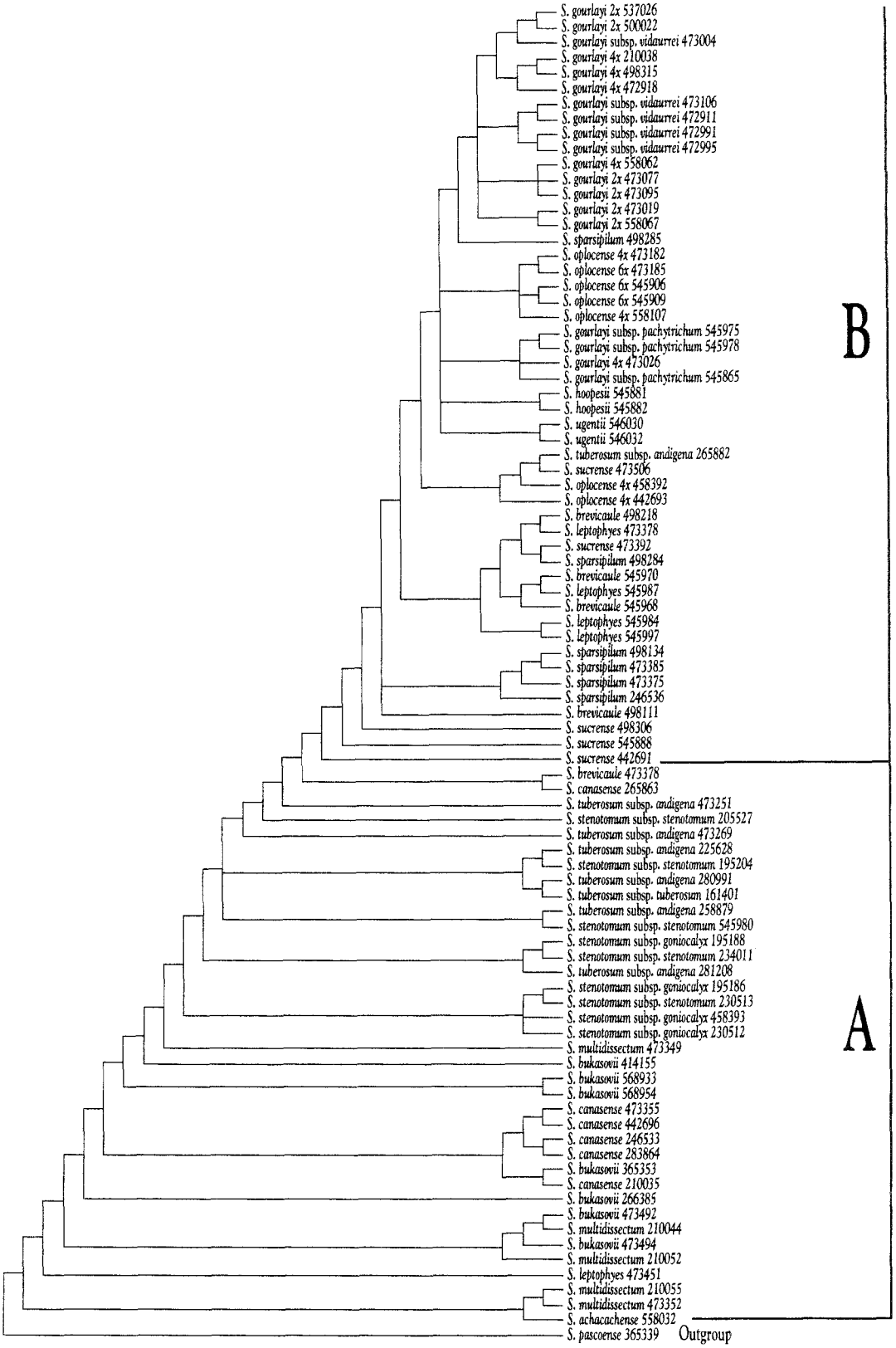

Fig. 5. Strict consensus cladogram of 88 trees of 2578 steps based on the total evidence combined RFLP and RAPD data of the 88 accessions in common between studies. Vertical bars indicate groups that correspond to those in Figs. 2–4

potato RFLP probes resulted in 4.1 bands per probe, not significantly different ($p = 0.19$; overall 4.4 bands were scored per probe). The RFLP data sets generated from mapped probes versus unmapped probes are correlated at 0.76. All correlations were significant ($p < 0.001$).

Testing hybridization hypotheses. None of the putative parental taxa involved in hybrid hypotheses shows species-specific bands. *Solanum tuberosum* subsp. *andigena* and one of its putative parents *S. stenotomum* are placed in grade A, whereas the other putative parent *S. sparsipilum*, which had four of five accessions form a distinct clade, is placed in clade B. Since *S. stenotomum* cannot be distinguished, these data cannot address the question of hybrid origin. *Solanum oplocense*, likewise, has no species-specific bands, and was not consistently distinguished from its putative hybrid *S. sucrense*. With these data the hybrid origin can neither be supported nor contradicted.

Discussion

Congruence among data sets and data analyses. Both RAPDs and RFLPs have the advantage of providing corroborative evidence to morphological data because they provide large numbers of discrete, and potentially taxon-specific characters. For interspecific comparisons, comigrating RAPDs may not always represent homologous characters (Smith & al. 1994, Thorman & al. 1994, Rieseberg 1996). Because homology is a function of taxonomic distance (Thormann & al. 1994, Rieseberg 1996), most RAPD bands should be homologous within the low taxonomic level of our study. Concordance between RFLPs and RAPDs have been shown in many studies (Halward & al. 1992, Wilkie & al. 1993, Williams & St. Clair 1993, Dos Santos & al. 1994, Hilu & Stalker 1995, Prince & al. 1995, Shanmukhaswami & al. 1995, Stammers & al. 1995, Dawson & al. 1996, Spooner & al. 1996). Loarce & al. (1996) found RFLP data to be in better agreement with known pedigree than RAPD data among rye cultigens.

Parsimony analysis may not show consistent results of gene trees below the species level (Baum & Donoghue 1995, Maddison 1995). Hybrids can occur between closely related species and not radically disrupt the phylogeny (Mcdade 1992, 1995). Several studies have implemented parsimony analysis on RFLP or RAPD data sets with results concordant with previous phylogenies (Van Heusden & Bachman 1992, Van Buren & al. 1994, Bark & Havey 1995, Brummer & al. 1995, Catalan 1995, Margale & al. 1995, White & al. 1995). RAPD based cladistic analysis gave better resolution than internal transcribed spacer of ribosomal genes (ITS) and morphology at the intraspecific level in *Silene* (Oxelman 1996). Our RFLP and RAPD results are concordant in a very weakly supported clade B (containing four previously presumed outgroup taxa) and basal grade A, but there is no concordance of topology of taxa within these.

Some reasonable degree of congruity is essential for combining data in a total evidence analysis. Congruity can be measured by distance method (Heun & al. 1994, Spooner & al. 1996), and topology methods (I_{MF}; Mickevich & Farris 1981, Swofford 1991). Combining data sets has been used to explore the congruity among phylogenies without explicit tests of incongruity. Olmstead & Sweere (1994) combined chloroplast DNA restriction site mapping with sequences from

*ndh*F and *rbc*L in *Solanaceae* into a total evidence data set. This resulted in a more resolved total evidence tree, as each individual data set did not have enough characters to resolve portions of the tree. KIM & JANSEN (1994) found almost twice the character incongruence with I_M than I_{MF} (22.5%, 13.3%) in *Krigia* among morphology, chloroplast DNA, ribosomal DNA and ITS data. BRUNEAU & al. (1995) used I_{MF} and other incongruity tests among chloroplast DNA, morphology and isozyme data of *Solanum* sect. *Lasiocarpa* and found that methods of testing incongruence differed in their evaluations but that the total evidence data set revealed the best resolution.

The I_M and I_{MF} topology-based indices for our data show comparatively good concordance of RFLP and RAPD trees (17.5%, 9.5%, respectively). The Spearman-rank distance method shows higher correlation of RFLP and RAPD results than either to the morphological results.

Our results support multiple genes to aid exploration of stable clades in a taxonomically complex group as an aid to discover apparent spurious relationships below the boundary of species. The combination of studying many loci from different data sets is a reciprocally illuminating approach to discovering clades that are stable. The level at which stable clades can be identified can be interpreted as the reticulation/divergence boundary, and putatively the boundary among species. Phylogenetic studies at or below the species level typically will require more gene tree investigations, of unlinked loci, in order to explore the boundary of reticulation and divergence, than will studies above the species level.

Paraphyly of the *S. brevicaule* complex. Cladistic analysis of the RFLP and RAPD data are concordant with the morphological phenetic analyses (VAN DEN BERG & al. 1998) in separating the *S. brevicaule* complex into 1) the northwestern Bolivian and Argentinean taxa (clade B), and 2) the Peruvian and adjacent northwestern Bolivian accessions with most of the cultigens (grade A). In all RFLP analyses, clade B consistently contains previously presumed outgroup taxa *S. chacoense*, *S. fendleri*, *S. vernei*, and *S. verrucosum*. The RAPD analysis did not examine these species, so paraphyly cannot be addressed with these data.

Similar, but smaller scale RFLP phylogenetic studies of DEBENER & al. (1990) and BONIERBALE & al. (1995) defined a clade A consisting only of Peruvian members of the *S. brevicaule* complex with cultigens, a clade B containing Bolivian and Argentinean members of the complex and other species not previously thought to be part of the complex, and outgroups. An Amplified Fragment Length Polymorphism (AFLP) study of six taxa and 12 populations of *S. brevicaule* and other taxa (KARDOLUS & al. 1997) likewise supported a paraphyletic *S. brevicaule* complex. Chloroplast DNA studies (HOSAKA 1995, SPOONER & CASTILLO 1997) failed to provide sufficient resolution to consistently differentiate these groups.

Our study highlights the importance of thorough taxonomic sampling. *Solanum gourlayi* subsp. *gourlayi* (2x, 4x) and *vidaurrei* form a distinct clade in the RAPD analysis but not in the RFLP analysis. If only the taxa in the RAPD analysis had been included in the RFLP analyses, two distinct clades would have been found correlating with a Peruvian/cultivated clade A and the Bolivian/Argentinean clade B (MILLER 1997). In that situation an incorrect conclusion of species boundaries would have been recognized.

Application of species theory. Phylogenies are reconstructions of histories based on characters. In the absence of recombination, different parts of a genome should reveal similar phylogenetic information above the species level. Recombination exchanges genetic information, causing different phylogenetic histories for different genes (recombination units) to be maintained within a single plant (MADDISON 1995), and may produce homoplasy in a cladistic analysis. For this reason, defining the level reticulation and divergence is crucial in the defining genealogical species.

Much current debate centers around applicability of appropriate data and analytical method to investigate the boundary of reticulation and divergence, at putative species level (BAUM & DONOGHUE 1995, MADDISON 1995). This study was initiated using the species designations of *Solanum* taxonomists. However, the present data clearly fails to support most of these species and infers reticulation or rampant parallel evolution of morphological traits within grade A and clade B.

The Phylogenetic Species Concept (PSC) of NIXON & WHEELER (1990) defines species based on diagnostic characters uniting a clade of populations and does not search for monophyly. They consider their concept inapplicable below the species level. Species are defined as the "smallest diagnosable units," based on non-overlapping character states (LUCKOW 1995). In this context only one morphological species would be recognized in the *S. brevicaule* complex because they are all distinguished only by overlapping ranges of character states. The molecular data, like the morphological data, fail to support species by species-specific bands.

An alternate species concept, The Genealogical Species Concept (GSC) attempts to discover monophyletic lineages. BAUM & DONOGHUE (1995) recognized that below the species level, reticulation can produce separate gene trees for the same study organisms. They propose a search for the boundary of reticulation and divergence by a coalescence approach that searches for concordance of cladistic results among different data sets.

An alternative apospecies/plesiospecies concept also searches for monophyletic species, but argues that some good morphological species will have monophyletic molecular support (apospecies) but that remnant plesiomorphic populations may be recognized as plesiospecies (OLMSTEAD 1995). Similar ideas were addressed by RIESEBERG & BROUILLET (1994).

An alternative non-topological approach is a gene pool classification (DOYLE 1995). Taxa are based on shared alleles at loci, and groups are defined by shared gene pools. This approach does not rely on relationships inferred from cladistic analyses. Strict adherence to this concept with our RFLP and RAPD data would define one gene pool for all of sect. *Petota* here examined, including the outgroups.

We interpret our results to support a single highly polymorphic species *S. brevicaule*, that exhibits a geographical component to the variability (Peru and immediately adjacent Bolivia and Argentina) as evidenced by both morphological and molecular data sets. It is impossible to consistently and reliably use morphology for practicable taxonomic separation of these geographical groups. The southern group contains morphologically distinguishable apospecies *S. chacoense*, *S. fendleri*, *S. vernei*, and *S. verrucosum.*

Origin of the cultivated potato. HAWKES (1990) hypothesized that the diploid cultivated potato species, *S. stenotomum*, which grows around Lake Titicaca on the border of Peru and Bolivia, as the original cultivated potato. He further hypothesized that the wild species *S. leptophyes* was the likely progenitor of *S. stenotomum*. Earlier, HAWKES (1958) proposed both *S. canasense* and *S. leptophyes* as possible progenitors of *S. stenotomum*. HAWKES (1990) hypothesized hybridization after domestication between *S. stenotomum* and *S. sparsipilum* to form an amphidiploid *S. tuberosum* subsp. *andigena*.

HOSAKA & HANNEMAN (1988) and HOSAKA (1995) used chloroplast DNA to examine origins of cultivated taxa. Seven of the eight wild taxa they examined had more than one chloroplast DNA type and these chloroplast types did not consistently define taxa. HOSAKA (1995) found that most of the accessions of *S. bukasovii*, *S. candolleanum*, *S. canasense*, and *S. multidissectum* had chloroplast DNA types similar to the cultigens *S. stenotomum* and *S. tuberosum* subsp. *andigena* (grade A), while *S. brevicaule* and *S. sparsipilum* (clade B) generally lacked these chloroplast types. HOSAKA'S (1995) data, as our data, suggest multiple origins and/or hybridization of cultivated taxa with wild species.

Investigations and hypotheses of cultivated species origins have been hampered by splintered taxonomic concepts in the *S. brevicaule* complex. Our new molecular data and morphological data (VAN DEN BERG & al. 1998) suggest that taxonomic concepts involving a single highly polymorphic species, with a geographic component to this variability (grade A and clade B) will be more appropriate for investigating the origins of the cultigens. Because 21 of the 22 examined accessions of cultigens are in Grade A (Peru and immediately adjacent northwestern Bolivia), it suggests that they originated from populations in this geographic area.

Summary and conclusions. Our molecular data concur with previous morphological data (VAN DEN BERG & al. 1998) in indicating that most of the 30 taxa of the *S. brevicaule* taxa are artificial. The patterns of morphological and molecular variability indicate two geographically-based groups of *S. brevicaule* s. l., one in Peru (grade A), including most of the accessions of the cultigens, and one from Bolivia and Argentina (clade B). Clade B includes phenetically distinct taxa not previously thought to be members of the complex (*S. chacoense*, *S. fendleri*, *S. vernei* and *S. verrucosum*). However, these geographic subsets of the *S. brevicaule* complex s. str. (exclusive of *S. chacoense*, *S. fendleri*, *S. vernei* and *S. verrucosum*) are distinguishable morphologically only with the aid of computer-assisted multivariate analyses, and in practicable application only *S. oplocense* can be distinguished with difficulty.

The elucidation of species boundaries and relationships at these taxonomic levels is potentially confounded by hybridization, lineage sorting, recent evolution, and spread of cultigens and associated weeds by humans that have disrupted formerly distinct taxa and habitats. These data suggest that the ultimate taxonomic resolution to this problem will be lumping all taxa into *S. brevicaule* s. l. as a plesiospecies, and the recognition of separate apospecies such as *S. chacoense*, *S. fendleri*, *S. oplocense*, *S. vernei* and *S. verrucosum*. We are continuing to study these species with additional molecular data and a replicated morphological study in Peru to investigative environmental influences. We think that these studies will

result in synonymy of all clade B and grade A taxa, exclusive of the apospecies mentioned above. This solution will appear extreme only in reference to the highly splintered and impractical current taxonomy.

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