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R.G. van den Berg · G.J. Bryan · A. del Rio
D.M. Spooner

Reduction of species in the wild potato Solanum section *Petota* series *Longipedicellata*: AFLP, RAPD and chloroplast SSR data

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Abstract Species boundaries were assessed with three molecular markers [AFLPs, RAPDs and chloroplast simple sequence repeats (cpSSRs)] for all six species of wild potatoes (*Solanum* section *Petota*) assigned to ser. *Longipedicellata*: *Solanum fendleri*, *S. hjertingii*, *S. matehualae*, *S. papita*, *S. polytrichon* and *S. stoloniferum*. These tetraploid (2n = 4*x* = 48) species grow in the southeastern United States (*S. fendleri*) and Mexico (all six species), and a recent morphological analysis supported only three species: (1) *S. polytrichon*, (2) *S. hjertingii* (including *S. matehualae*) and (3) *S. stoloniferum* (including *S. fendleri* and *S. papita*). We analyzed all six species of ser. *Longipedicellata* (tetraploid) and also analyzed diploids in ser. *Bulbocastana*, ser. *Pinnatisecta*, ser. *Polyadenia* and ser. *Tuberosa*; tetraploids in ser. *Acaulia* and hexaploids in ser. *Demissa*. Concordant with morphological data, AFLP and RAPD results support the synonymy of *S. hjertingii* and *S. matehualae*, and completely intermix *S. papita* and *S. fendleri*. However, accessions of *S. stoloniferum* have a tendency to cluster but with exceptions, and *S. polytrichon* is completely intermixed with *S. fendleri* and *S. papita*. The cpSSRs fail to distinguish any of the species in ser. *Longipedicellata*. Combined morphological and molecular data support only two species in ser. *Longipedicellata*: *S. hjertingii* and *S. stoloniferum*.

Keywords AFLP · Chloroplast simple sequence repeat · Potato · RAPD · *Solanum* section *Petota* series *Longipedicellata* · SSR

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R.G. van den Berg (✉)
Biosystematics Group, Department of Plant Sciences,
Wageningen University, 6703 BL Wageningen, The Netherlands
e-mail: ronald.vandenberg@wur.nl
Tel.: +31-(0)-317483377, Fax: +31-(0)-317484917

G.J. Bryan
Scottish Crop Research Institute,
Invergowrie, Dundee, UK, DD2 5DA, UK

A. del Rio
Department of Horticulture, University of Wisconsin,
1575 Linden Drive, Madison, Wisconsin 53706-1590, USA

D.M. Spooner
Vegetable Crops Research Unit, USDA,
Agricultural Research Service, Department of Horticulture,
University of Wisconsin, 1575 Linden Drive, Madison,
Wisconsin 53706-1590, USA

Introduction

Solanum L. sect. *Petota* Dumort., the potato and its wild relatives, is distributed from the southwestern United States to southern Chile, with a concentration of diversity in the Andes. Hawkes (1990) recognized 232 species, partitioned into 21 series. Spooner and Hijmans (2001) updated this to 199 species, considering taxonomic changes since 1990. *Solanum* ser. *Longipedicellata* Buk. currently contains six species, distributed from the southwestern United States to southern Mexico (*S. fendleri* A. Gray, *S. hjertingii* Hawkes, *S. matehualae* Hjert. and T.R. Tarn, *S. papita* Rydb., *S. polytrichon* Rydb., and *S. stoloniferum* Schltdl.). All are tetraploid (2n = 4*x* = 48), are freely intercrossable with each other, have a strong EBN (Endosperm Balance Number)-based biological isolating mechanism from members of most other series, and have been postulated to possess AABB genomes (Hawkes 1990).

Spooner et al. (2001a) studied the morphological support for all six species of ser. *Longipedicellata*. They discovered that putative "species-specific" morphological characters were shared among many species in the series, and even with some species in other series. The most liberal interpretation of the morphological data supported only three species: (1) *S. polytrichon*, (2) *S. hjertingii* (including *S. matehualae*), and (3) *S. stoloniferum* (including *S. fendleri* and *S. papita*). The present study uses three molecular markers to further test the species boundaries within ser. *Longipedicellata* using the same accessions as in the morphological study.

Table 1 Accessions of *Solanum* section *Petota* examined. The species name is followed by the six-digit plant introduction number, the C-number from the Centre for Genetic Resources The Netherlands, or the B-number from the Braunschweig Genetic Resources Centre, Germany; then the accession number corresponding to Figs. 2 and 3; then a map locality corresponding to Figs. 1–3. All accessions were examined for AFLPs (A), RAPDs (R) and cpSSRs (C), unless noted otherwise

Series *Longipedicellata*

S. fendleri 458418, 1,1; 458422, 2,1; 458420, 3,3; 275161, 4,4; 275156, 5,5; 498001, 6,6; 498240, 7,6; 497994, 8,7; 497998, 9,9; 497995, 10,10; 558398, 11,11; 558397, 12,12; 262895, 13,13; 283102, 14,14; 558395, 15,15; *S. hjertingii* 498019, 16,21; 545713, 17,21; 251067, 18,22; *S. matehualae* 498050, 19,23; *S. papita* 283101-R, 20,8; 249929-R, 21,17; 275227, 22,17; 498035-R; 23,18; 545732-R, 24,18; 545726, 25,19; 545723-R, 26,20; 498028, 27,24; 498030, 28,24; 545724, 29,24; 498027, 30,25; 275229, 31,26; *S. polytrichon* 255547-R, 32,17; 184770-R; 33,26; 545780, 34,27; 545786-R, 35,29; 498276, 36,35; 558454, 37,35; 255546, 38,37; 338620, 39,41; *S. stoloniferum* 283108, 40,14; 545740-R, 41,20; 545793, 42,28; 255534, 43,33; 239410, 44,39; 558467, 45,41; 186555, 46,42; 161178, 47,43; 186544, 48,43; 365399, 49,43; 498287, 50,47a; 558475, 51,47b; C18332-A,S, 52, unknown locality

Members of other series

S. acaule 472735-R, 53; *S. albicans* 230494-R, 54; *S. avilesii* 498091, 55; *S. brachycarpum* C17721, 56; C18347-A,S, 57; C20561-A,S, 58; *S. berthaultii* 283069, 59; *S. bulbocastanum* 255516, 60; *S. brachistotrichium* 320265, 61; *S. demissum* C17787-A,S, 62; C17788-A,S, 63; 205514, 64; C17817, 65; *S. guerreroense* C18920, 66; B7186, 67; *S. hougasii* 161174-R, 68; 161726-R, 69; *S. iopetalum* C20572, 70; 275182-R, 71; *S. piurae* 310997, 72; *S. polyadenium* 347769-R, 73; *S. schenckii* C18361-A,S, 74; *S. verrucosum* 310966-R, 75; 498061, 76; 545745, 77; 558457, 78

Materials and methods

Species

We analyzed a total of 78 accessions, and mapped members of ser. *Longipedicellata* to 36 generalized geographic regions (Table 1, Fig. 1). Fifty six of these 78 accessions were analyzed in common with AFLP, RAPD and cpSSR data. For efficient comparison to the morphological study of Spooner et al. (2001a) we maintain the same map numbering scheme of that paper. We analyzed all six species of ser. *Longipedicellata* (*S. fendleri* 15 accessions, *S. hjertingii* 3, *S. matehualae* 1, the only accession available of this rare species, *S. papita* 12, *S. polytrichon* 8, and *S. stoloniferum* 13). We also analyzed diploid species in ser. *Bulbocastana* (Rydb.) Hawkes (*S. bulbocastanum* Dunal 1), ser. *Pinnatisecta* (Rydb.) Hawkes (*S. brachistotrichium* (Bitter) Rydb. 1), ser. *Piurae* Hawkes (*S. piurae* Bitter 1), ser. *Polyadenia* Correll (*S. polyadenium* Greenm. 1), ser. *Tuberosa* (Rydb.) Hawkes (*S. avilesii* Hawkes and Hjert. 1, *S. berthaultii* Hawkes 1, *S. verrucosum* Schltdl. 4); tetraploid and hexaploid species in ser. *Acaulia* Juz. (*S. acaule* Bitter 1, *S. albicans* 1) and hexaploid species in ser. *Demissa* Buk. (*S. brachycarpum* Correll 3, *S. demissum* Lindl. 4, *S. guerreroense* Correll 2, *S. hougasii* Correll 2, *S. iopetalum* (Bitter) Hawkes 2, *S. schenckii* Bitter 1). Cladistic studies of Spooner and Castillo (1997) support *S. brachistotrichium*, *S. bulbocastanum*, *S. piurae*, and *S. polyadenium* as outgroups.

Most accessions were from the National Research Support Program-6 (NRSP-6; Bamberg et al. 1996). They represent the maximum geographic distribution available from genebank collections (Fig. 1) and nearly the entire geographic ranges for these species. Vouchers are deposited at NRSP-6 Sturgeon Bay, Wisconsin. Identifications of these accessions were provided by visiting taxonomists to NRSP-6 to identify living representatives.

DNA isolation and purification

All molecular marker studies used DNA aliquots from the same single extraction of a single individual per accession. Fresh leaves were collected from 2-month-old plants and DNA was extracted following the procedure of Doyle and Doyle (1987), with purification on CsCl/ethidium bromide gradients.

AFLP primer selection and amplification

Two AFLP primer combinations, chosen based on successful use in wild potatoes, were used to generate AFLP fragments (Kardolus 1998). One set of fragments (primers *Eco*RI+AAC/*Mse*I+CAC), was generated with the AFLP® plant mapping kit (Perkin Elmer Applied Biosystems) following the manufacturer's directions. KeyGene Marker Systems (Wageningen, The Netherlands) generated another fragment set (*Eco*RI+ACA/*Mse*I+CAC) using procedures described in Vos et al. (1995).

RAPD primer selection and amplification

A total of 21 10-mer RAPD primers (Operon Technologies, Almeda, Calif.) were selected based on clearly discernible polymorphic bands: OPA-2, OPA-4, OPAA-1, OPAA-10, OPAA-14, OPAA1-6, OPAC1-5, OPAC-9, OPAF-3, OPAG-4, OPAG-9, OPAI-10, OPAJ-1, OPD-1, OPE-18, OPG-6, OPM-12, OPM-2, OPQ-17, OPU-3 and OPV-8. Procedures follow Spooner et al. (2001b).

Chloroplast DNA SSR amplification

Ten cpSSR primer pairs were used in this study (NTCP3, NTCP4, NTCP6, NTCP7, NTCP8, NTCP9, NTCP11, NTCP12, NTCP18 and NTCP39) and were selected on the basis of the high levels of intra- and inter-specific polymorphism shown in Bryan et al. (1999). Genomic sequences were amplified following procedures in Bryan et al. (1999).

Data analysis

All data were analyzed with NTSYS-pc® version 2.02k (Rohlf 1992). The AFLPs and RAPDs are dominant markers, for which Jaccard's similarity coefficient is appropriate. Clustering was performed using the unweighted pair-group method (UPGMA). Cophenetic correlation coefficients were calculated to measure distortion between the similarity matrices and the resulting phenograms (Sokal 1986).

The cpSSRs were analyzed once as alleles arranged by relative size within each of the ten primers (ten characters with ordered multiple states), and also as presence/absence of each allele (44 0/1 characters). The ten-character dataset similarity was calculated using a city block distance measure (MANHAT), and for the 44 character dataset by a simple matching coefficient (SM). Clustering was performed using UPGMA.

Fifty six of the 78 accessions were analyzed in common with all three marker systems (Table 1), and separate analyses were performed on these smaller datasets as described above. Cophenetic value matrices were generated from all three of the resulting phenograms. The Mantel matrix correspondence test was used to

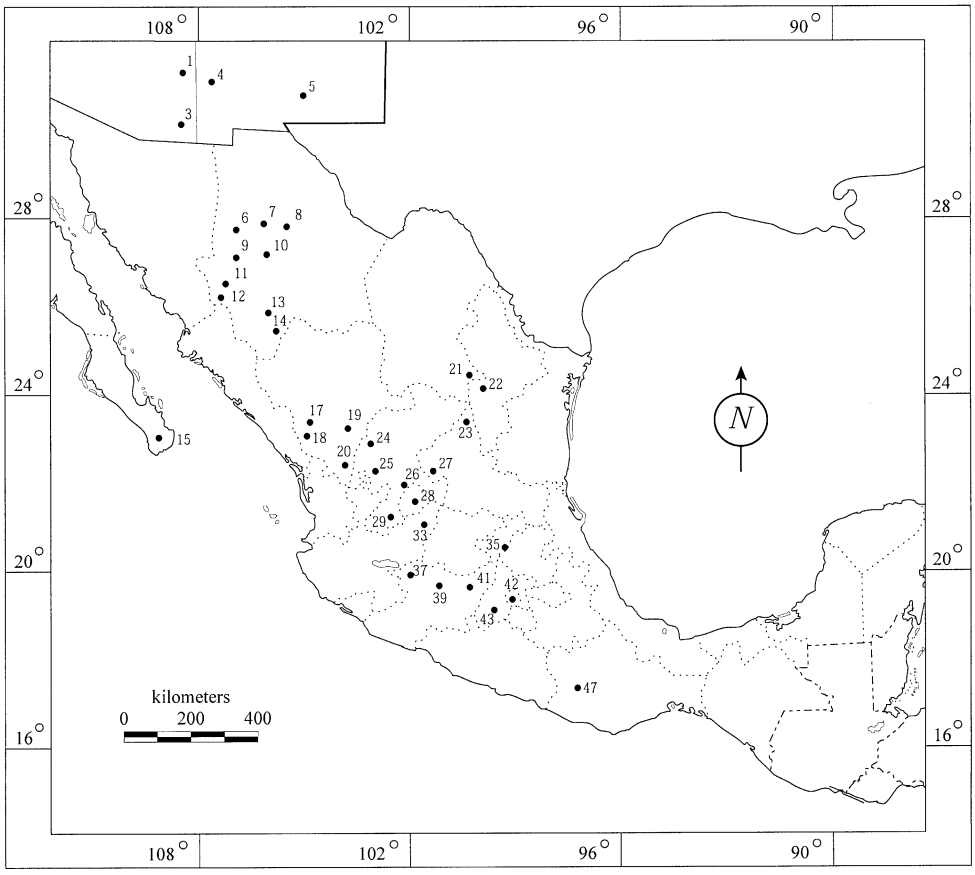

Fig. 1 Map showing the 36 generalized areas of the accessions of *S. fendleri*, *S. hjertingii*, *S. matehualae*, *S. papita*, *S. polytrichon* and *S. stoloniferum* examined in this study. For efficient comparison to the morphological study of Spooner et al. (2001a) we maintain the same map numbering scheme of that paper. The accessions of other species are not mapped. Numbers are cited as generalized map areas in Table 1 and Figs. 2 and 3

compare the cophenetic matrices and the similarity matrices among themselves. Combined datasets (AFLP + RAPD and AFLP + RAPD + cpSSR) of the 56 common accession were analyzed with Jaccard's similarity coefficient (even though Jaccard's is not an optimal similarity measure for cpSSRs).

Phylogenetic reconstructions on all datasets also were performed using PAUP version 4.0b8 (Swofford 2001), using Wagner parsimony. *S. brachistotrichium*, *S. bulbocastanum*, *S. palustre* (only with RAPDs) and *S. piurae* were used as outgroups, following the results of Spooner and Castillo (1997). To find multiple tree islands, we used a four-step search strategy following Olmstead and Palmer (1994). We also ran cladistic analyses as an unrooted network of just tetraploid members of ser. *Longipedicellata* because some of the diploids could be B-genome contributors to the allotetraploids, and mixing ploidy levels in these hybrids could complicate cladistic interpretations.

Results

The AFLP and RAPD phenograms, as separate or combined AFLP + RAPD analyses of a reduced dataset of 56 common accessions, had very high cophenetic correlation coefficients (r = >0.96), indicating excellent fits of the similarity matrices to the resulting phenograms, while all those with cpSSRs were lower (0.80–0.89). Similarly, the topologies of the AFLP and RAPD phenograms were similar to each other by inspection, while both were very different to the cpSSR phenogram. This discordance of cpSSR results also is evident by the r values comparing distance matrices of different data sets holding common accessions to each other. All comparisons involving cpSSRs were between 0.44 and 0.57, while those of AFLP to RAPD were 0.64.

The two cpSSR phenograms produced with the simple matching similarity coefficient (Fig. 2) and with the city block distance measure (data not shown) place the outgroups *Pinnatisecta, Piurana* and *Polyadenia* as generally basal. However, they completely separate accessions of other series.

The combined AFLP + RAPD phenetic and cladistic results are similar to each other. Cladistic results produced four most-parsimonious 1,414-step trees with a consistency index of 0.22 and a retention index of 0.50. A strict consensus tree of these four trees (Fig. 3) forms a relatively well-supported clade (72% bootstrap value) of all members of ser. *Longipedicellata* to also include *S. avilesii* 55 and *S. guerreroense* 66 from other series. *S. hjertingii* and *S. matehualae* form a clade with moderate bootstrap value (59%). Eight of the 11 accessions of *S. stoloniferum* form a clade (61% bootstrap) sister to *S. hjertingii* + *S. matehualae*. Fourteen of the 15 accessions of *S. fendleri* form a barely supported clade (<5% bootstrap) but intermixed with one accession of *S. stoloniferum* and two of the five accessions of *S. polytrichon*. All seven accessions of *S. papita* form a barely supported clade (<5% bootstrap), but with the non-ser. *Longipedicellata* members *S. avilesii* and *S. guerreroense*, and with an accession of *S. fendleri*. An unrooted

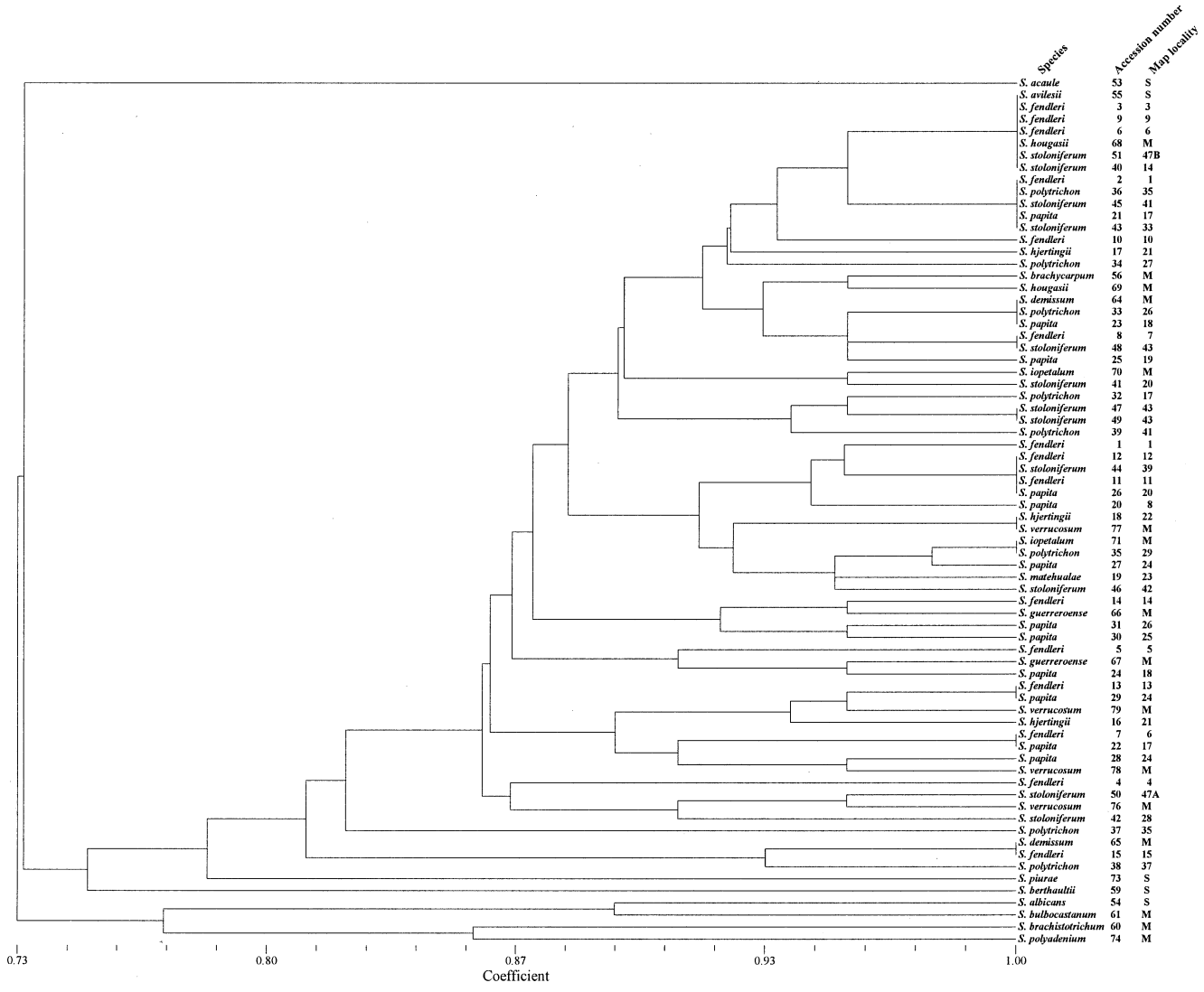

Fig. 2 UPGMA phenogram (simple matching distance coefficient) of the entire cpSSR dataset. Accession numbers and map localities as in Table 1

analysis of just tetraploid members of ser. *Longipedicellata* provides clades very similar to Fig. 3 and does not change our conclusions regarding ser. *Longipedicellata*.

Discussion

This study was intended to see if there was molecular support for the six widely accepted wild potato species in ser. *Longipedicellata*. Earlier authors (Correll 1962; Hawkes 1990) have noted the extreme difficulty in circumscribing the species of ser. *Longipedicellata*. Morphological results (Spooner et al. 2001a) suggested that there were at best only three species in series *Longipedicellata*: (1) *S. polytrichon*, (2) *S. hjertingii* (including *S. matehualae*), and (3) *S. stoloniferum* (including *S. fendleri* and *S. papita*). That morphological study showed these species to be supported only by a series of character states exhibiting overlapping ranges with other species (polythetic support, Sokal and Sneath 1963). This was especially true for *S. polytrichon*, supported only by a canonical variate analysis, but not by a principal components analyses that intermixed *S. polytrichon* with many other species. Putative *S. polytrichon* diagnostic characters of spreading pubescence, enlarged terminal leaflets and white corollas varied tremendously in this species (Spooner et al. 2001a, Figs. 3, 4), and it is impossible to unambiguously identify many specimens. It is untenable to continue to recognize all six species of ser. *Longipedicellata* in light of these results.

The chloroplast DNA microsatellite results are completely discordant with AFLP and RAPD results and greatly intermix species. Different molecular results of the same taxa are rarely completely concordant (Wendel and Doyle 1998), and microsatellite results are frequently very discordant with other molecular data (Milbourne et al. 1997; Russell et al. 1997). The great cpSSR discordance could be a function of the low numbers of markers (10 as scored as alleles in a locus, 44 when scored

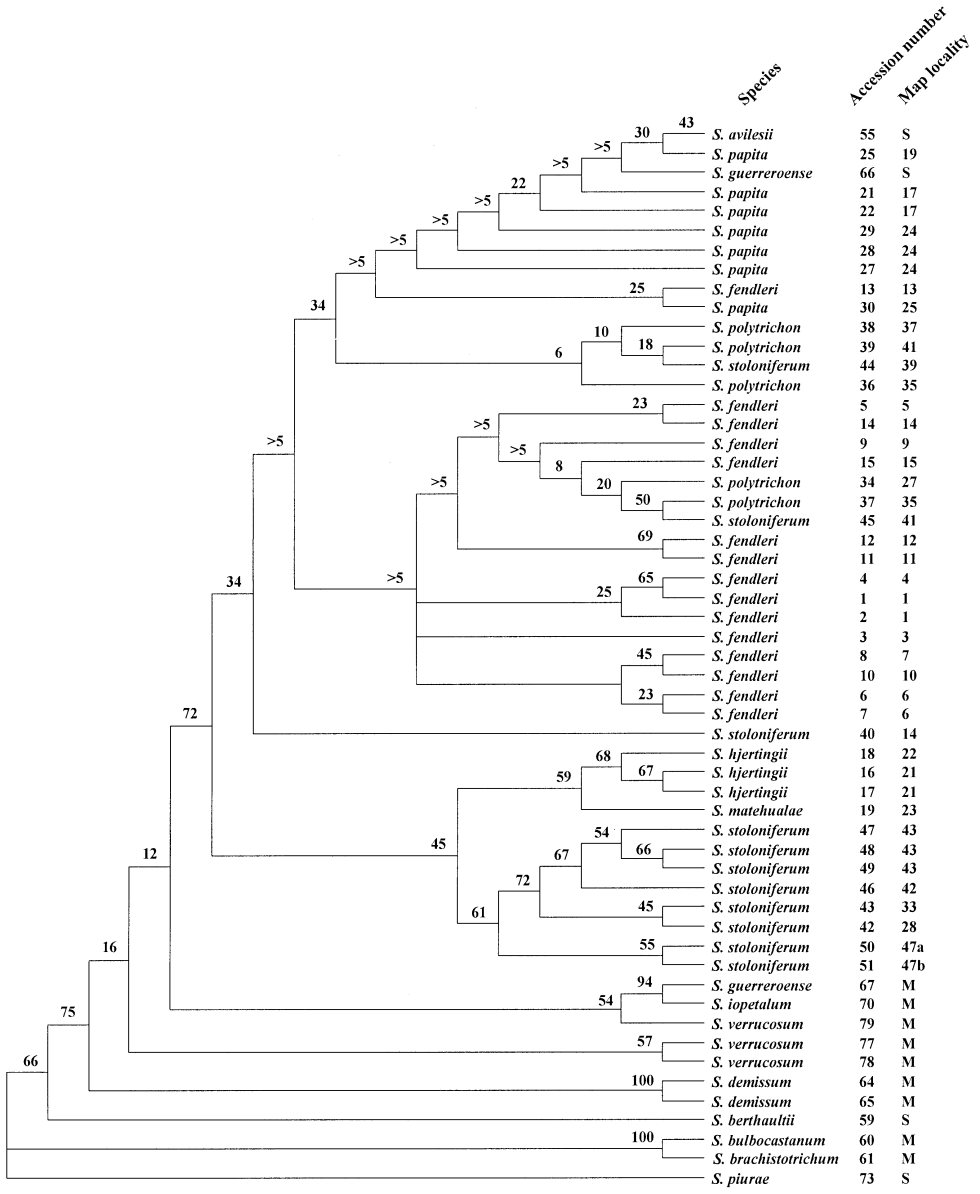

Fig. 3 The strict consensus tree of four equally parsimonious 1,414-step Wagner trees, with bootstrap values

as separate characters, vs 79 for RAPDs and 419 for AFLPs). Another possible cause for discordance of cpSSR results may be the predominantly maternal inheritance of cpDNA in *Solanum* (Corriveau and Coleman 1988) and a separate history of chloroplasts because of hybrid origins (Wendel and Doyle 1998). If these cpSSR data are providing a true phylogenetic signal, it suggests a history of extensive separate and independent origins of members of ser. *Longipedicellata*, a hypothesis that needs further testing with genome-specific markers, as has been done with allotetraploids of *Elymus* with single-copy nuclear-encoded "waxy" gene sequences (Mason-Gamer 2001).

Many accessions of *S. stoloniferum* cluster in AFLP and RAPD analysis except for *S. stoloniferum* accessions 40, 44 and 45. One of the three accessions of *S. stoloniferum* (40) was collected in northern Mexico, and could be a misidentified *S. fendleri*, as the former was believed to occur in central to southern Mexico and the latter in northern Mexico. Our inspection of living plants of accessions 40, however, show it not to possess any of the clear traits previously used to distinguish *S. fendleri* from *S. stoloniferum* (Hawkes 1990). Similarly, our inspection of living plants of accessions 44 and 45 show them not to have any of the obvious traits previously used to distinguish *S. fendleri*. All accessions of *S. papita* form a clade, but with hardly any bootstrap support, and intermixed with three other species.

Solanum hjertingii (to include *S. matehualae*) is embedded in other members of ser. *Longipedicellata*. Recently, Olmstead (1995) argued that geographically localized models of speciation typically produce a monophyletic daughter species and remnant paraphyletic progenitor species, and argued that a strict concept for monophyly fails for many species. Olmstead (1995) termed the former apospecies and the latter plesiospecies. Our recog-

nition of *S. hjertingii* is justified by its morphological distinctness (Spooner et al. 2001a), distinct range (in northwestern Mexico) and an apospecies concept.

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