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Taxonomy of North and Central American diploid wild potato (*Solanum* sect. *Petota*) species: AFLP data

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Abstract. *Solanum* section *Petota*, the potato and its wild relatives, includes about 200 wild species distributed from the southwestern United States to central Argentina and adjacent Chile, with about 30 species in North and Central America. The North/Central American region and the South American region all include diploids, tetraploids, and hexaploids. Chloroplast DNA restriction enzyme data from a prior study showed that 13 of the North/Central American species formed a clade containing only diploids, but there was low resolution within the clade. This Amplified Fragment Length Polymorphism (AFLP) study is conducted to provide additional resolution within the North/Central American diploids and complements the chloroplast results, and prior morphological results. Wagner parsimony and phenetic analyses mostly agreed with the morphological data in supporting currently recognized species except that they suggest that *S. brachistotrichium* and *S. stenophyllidium* are conspecific. Our new AFLP data, in combination with the cpDNA and morphological data, also support sister taxon relationships for the following diploid species from North and Central America: 1) *S. cardiophyllum* subsp. *ehrenbergii* and *S. stenophyllidium*, 2) *S. tarnii* and *S. trifidum*, 3) *S. jamesii* and *S. pinnatisectum*, 4) *S. lesteri* and *S. polyadenium*, and 5) *S. clarum* and *S. morelliforme*.

Key words: AFLP, molecular phylogeny, potato, *Solanum* sect. *Petota*, systematics, taxonomy.

There are approximately 30 species of wild potatoes in the United States, Mexico, and Central America (here referred to as North and Central America), out of the total of about 200 species in *Solanum* L. sect. *Petota* Dumort., that extends to south-central Argentina and adjacent Chile (Spooner and Hijmans 2001). The North/Central American region and the South American region include diploids, tetraploids, and hexaploids. The North/Central American diploids are classified in the series *Bulbocastana*, *Morelliformia*, *Pinnatisecta*, *Polyadenia* and *Tuberosa* (Hawkes 1990). This study considers the species boundaries and taxonomic relationships of these diploid species.

A phylogeny based on chloroplast DNA (cpDNA) restriction enzyme sites (Spooner and Sytsma 1992, Spooner and Castillo 1997) divide sect. *Petota* into four clades (Fig. 1): 1) the North/Central American diploid species, exclusive of *S. bulbocastanum*, *S. cardiophyllum*, and *S. verrucosum*, 2) *S. bulbocastanum* and *S. cardiophyllum*, 3) the South American series *Piurana* and some South

American species classified in other series, 4) all remaining South American species and the North/Central American polyploid species and the diploid species *S. verrucosum*. Clade 1 includes series *Pinnatisecta*, with internested series *Polyadenia*, *Bulbocastana* (*S. cardiophyllum*) and series *Morelliformia*, and clade 2 ser. *Bulbocastana* (*S. bulbocastanum*) and ser. *Pinnatisecta* (*S. cardiophyllum*).

The only North/Central American diploid species not part of clades 1 and 2 is *S. verrucosum*. This species shares morphology and crossability relationships with members of clade 4 and clearly is unrelated to the clade 1 and clade 2 diploids (Spooner and Sytsma 1992). For ease of discussion, the term "North/Central American diploids" is used in this paper to include all species from this region except *S. verrucosum*.

The separation of *S. bulbocastanum* and *S. cardiophyllum* from clade 1 species was unexpected based on crossing data (Magoon et al. 1958, Graham et al. 1959, Graham and Dionne 1961), immunological data (Gell et al. 1960), and morphological data (Hawkes 1990) data. Rodríguez and Spooner (1997) investigated *S. bulbocastanum* and *S. cardiophyllum* by including more accessions and all the subspecies of each species. The resulting tree

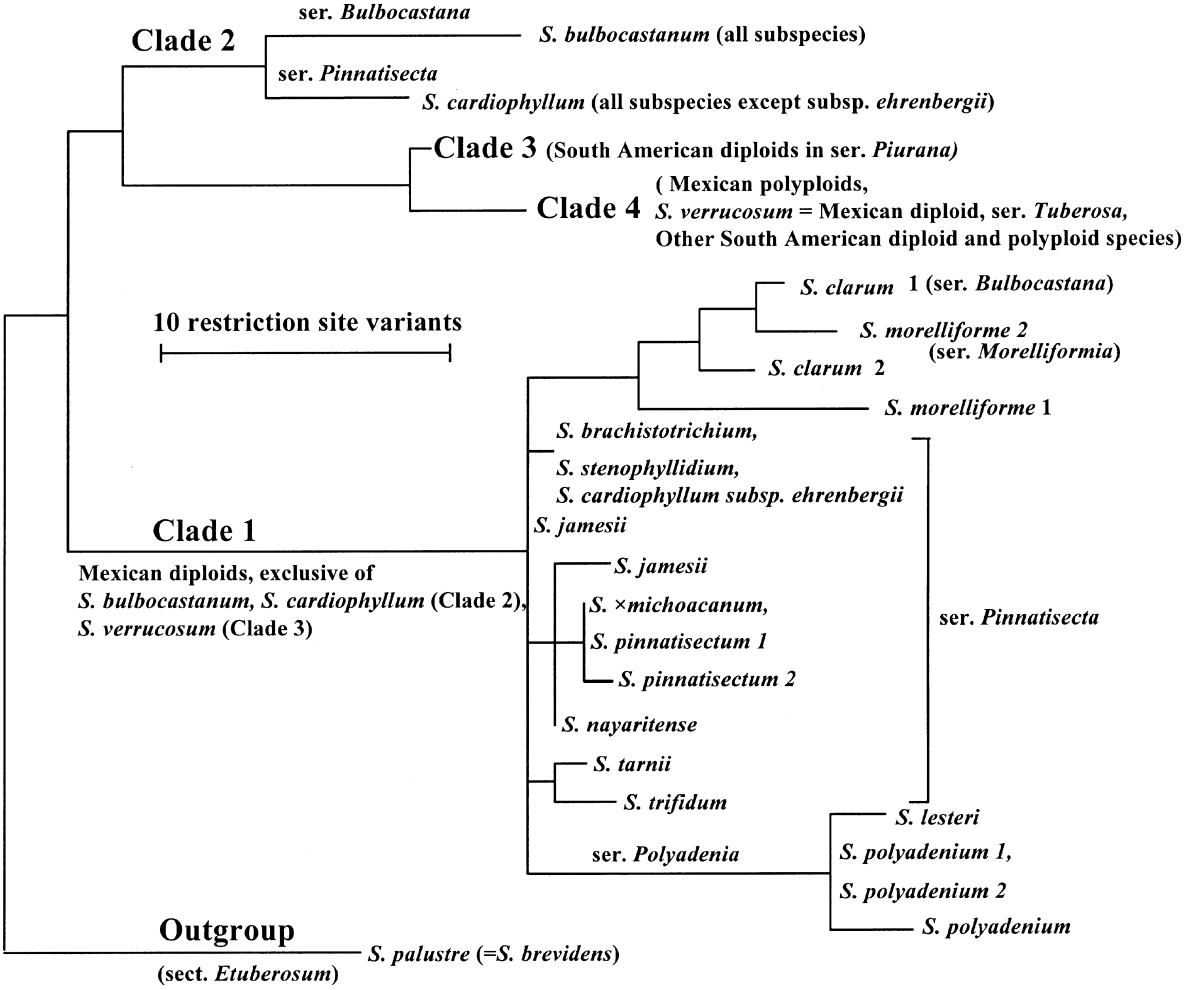

Fig. 1. Abstracted summary results of the chloroplast DNA restriction site analyses of Spooner and Sytsma (1992), Rodríguez and Spooner (1997), and Spooner and Castillo (1997)

maintains all the subspecies in clade 2 except *S. cardiophyllum* subsp. *ehrenbergii*, which is placed in the North/Central American diploid clade 1 (Fig. 1). Unpublished data from Spooner and collaborators of the internal transcribed spacer region of nuclear ribosomal DNA, and the GBSSI gene, however, show *S. bulbocastanum* and all subspecies of *S. cardiophyllum* to be sister taxa, showing a discordance of the cpDNA data and nuclear DNA data.

Lara-Cabrera and Spooner (in press) used morphology and nuclear DNA microsatellites developed for *S. tuberosum* to study the validity of the North/Central American diploid species in clade 1 and clade 2. They demonstrated that these microsatellite primers were not useful for this phylogenetic study, possibly because of divergence of priming sites that were developed for *S. tuberosum* of clade 4. The morphological data, however, provided good support for the North/Central American diploid species except that *S. brachistotrichium* and *S. stenophyllidium* cluster together (Fig. 2).

Our present study explores the use of another molecular marker, Amplified Fragment Length Polymorphisms (AFLPs; Vos et al. 1995), to study the status of species, and interrelationships, of the North/Central American diploids. The accessions of our present AFLP study are a subset of those from the morphological study (Lara-Cabrera and Spooner, in press), with only four exceptions (Table 1).

The AFLP technique combines restriction enzyme reactions with the Polymerase Chain Reaction (PCR), revealing high levels of polymorphism (Vos et al. 1995). The AFLP technique is becoming the technique of choice when there is no previous knowledge of sequence variation or developed molecular probes for a group. AFLPs have high reproducibility, providing advantages over other anonymous molecular markers like Random Amplified Fragment Length Polymorphisms (RAPDs), and are not as expensive as other techniques like nuclear Restriction Fragment Length Polymorphisms (RFLPs; Mueller and Wolfenbarger 1999). They have tremendous

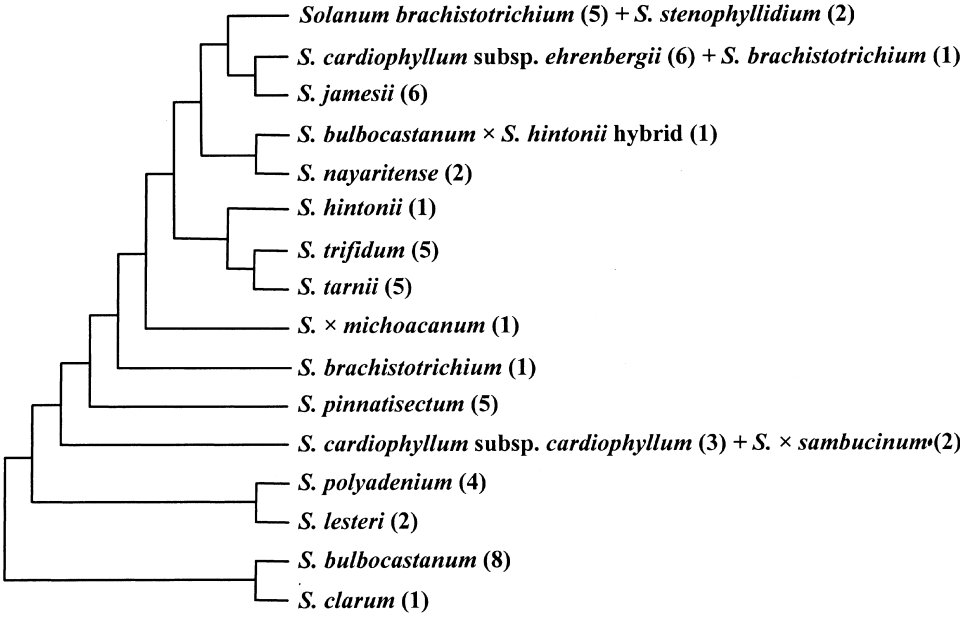

Fig. 2. Abstracted summary results of the morphological phenetic analyses of the North/Central American diploid species by Lara-Cabrera and Spooner (in press). The numbers of accessions analyzed per taxon are indicated in parentheses)

Table 1. Series affiliation within *Solanum* sect. *Petota*, PI (USDA Plant Introduction) number, and abstracted locality of the accessions examined in this study. Herbarium vouchers are deposited at PTIS. All of these accessions were studied in Lara-Cabrera and Spooner (in press) except the two outgroups (*S. etuberosum* and *S. palustre*), *S. morelliforme*, and the *S. bulbocastanum* × *S. cardiophyllum* hybrid

Series placement by Hawkes (1990)	Taxon	PI	Country: State or Department[a]
Bulbocastana (Rydb.) Hawkes	*Solanum bulbocastanum* Dunal subsp. *bulbocastanum*	275198	Mexico: México
	S. bulbocastanum subsp. *bulbocastanum*	275184	Mexico: Distrito Federal
	S. bulbocastanum subsp. *bulbocastanum*	275194	Mexico: Oaxaca
	S. bulbocastanum subsp. *dolichophyllum* (Bitter) Hawkes	498224	Mexico: Michoacán
	S. bulbocastanum subsp. *dolichophyllum*	545752	Mexico: México
	S. bulbocastanum subsp. *dolichophyllum*	255516	Mexico: Jalisco
	S. bulbocastanum subsp. *partitum* (Correll) Hawkes	558379	Mexico: Chiapas
	S. bulbocastanum subsp. *partitum*	275200	Guatemala: Huehuetenango
	S. clarum Correll	558382	Mexico: Chiapas
Morelliformia Hawkes	*S. morelliforme*	275218	Mexico: México
	S. morelliforme	498003	Mexico: México
Pinnatisecta (Rydb.) Hawkes	*S. morelliforme*	545774	Mexico: Chiapas
	S. brachistotrichium	498217	Mexico: Chihuahua
	S. brachistotrichium	497993	Mexico: Chihuahua
	S. brachistotrichium	545832	Mexico: Aguascalientes
	S. brachistotrichium	545815	Mexico: Aguascalientes
	S. brachistotrichium	255527	Mexico: Aguascalientes
	S. cardiophyllum Lindl. subsp. *cardiophyllum*	347759	Mexico: Puebla
	S. cardiophyllum subsp. *cardiophyllum*	283062	Mexico: State unknown
	S. cardiophyllum subsp. *cardiophyllum*	283063	Mexico: State unknown
	S. cardiophyllum subsp. *ehrenbergii* Bitter	279272	Mexico: Aguascalientes
	S. cardiophyllum subsp. *ehrenbergii*	184762	Mexico: Quéretaro
	S. cardiophyllum subsp. *ehrenbergii*	341231	Mexico: Jalisco
	S. cardiophyllum subsp. *ehrenbergii*	186548	Mexico: Zacatecas
	S. cardiophyllum subsp. *ehrenbergii*	255520	Mexico: San Luis Potosí
	S. cardiophyllum subsp. *ehrenbergii*	275213	Mexico: Quéretaro
	S. hintonii Correll	607880	Mexico: México
	S. jamesii Torr.	458425	USA: Arizona
	S. jamesii	564051	USA: Arizona
	S. jamesii	564049	USA: New Mexico
	S. jamesii	275169	USA: New Mexico
	S. jamesii	458423	USA: New Mexico
	S. jamesii	275172	USA: Arizona
	S. ×*michoacanum* (Bitter) Rydb. (putative origin is *S. bulbocastanum* × *S. pinnatisectum*)	558497	Mexico: Michoacán
	S. nayaritense (Bitter) Rydb.	545827	Mexico: Nayarit
	S. nayaritense	545820	Mexico: Zacatecas
	S. nayaritense	545825	Mexico: Zacatecas

Table 1 (continued)

Series placement by Hawkes (1990)	Taxon	PI	Country: State or Department[a]
	S. pinnatisectum Dunal	275234	Mexico: Jalisco
	S. pinnatisectum	184764	Mexico: Guanajuato
	S. pinnatisectum	275233	Mexico: Guanajuato
	S. pinnatisectum	275236	Mexico: Jalisco
	S. pinnatisectum	275230	Mexico: Quéretaro
	S. pinnatisectum	190115	Mexico: Michoacán
	S. bulbocastanum × *S. cardiophyllum* putative hybrid		Mexico: Jalisco
	S. ×*sambucinum* Rydb. (putative origin is *S. ehrenbergii* × *S. pinnatisectum*)	595478	Mexico: Quéretaro
	S. ×*sambucinum*	604209	Mexico: Guanajuato
	S. stenophyllidium Bitter	558460	Mexico: Jalisco
	S. tarnii Hawkes et Hjert.	498048	Mexico: Hidalgo
	S. tarnii	545808	Mexico: Hidalgo
	S. tarnii	545742	Mexico: Veracruz
	S. tarnii	498043	Mexico: Hidalgo
	S. tarnii	570641	Mexico: Hidalgo
	S. trifidum Correll	255542	Mexico: Michoacán
	S. trifidum	558478	Mexico: Michoacán
	S. trifidum	558480	Mexico: Michoacán
	S. trifidum	283104	Mexico: Jalisco
Polyadenia Bukasov	*S. lesteri* Hawkes et Hjert.	558434	Mexico: Oaxaca
	S. lesteri	442694	Mexico: Oaxaca
	S. lesteri	558435	Mexico: Oaxaca
	S. polyadenium Greenm.	498036	Mexico: Hidalgo
	S. polyadenium	347769	Mexico: Puebla
	S. polyadenium	347770	Mexico: Veracruz
Sect. *Etuberosum* (outgroup)	*S. etuberosum* Lindl.	558285	Chile: Santiago
	S. palustre Poepp.	558253	Chile: Los Lagos

[a]More complete locality data and a map of these accessions are found in Lara-Cabrera and Spooner (in press).

utility to provide more resolution than cpDNA restriction site data and nuclear ribosomal internal transcribed spacer data at the genus level (Despres et al. 2003, Beardsley and Olmstead 2003).

In the Solanaceae, Mace et al. (1999b) used AFLP data to study the genetic relationships among cultivated and wild eggplants (*Solanum* subg. *Leptostemonum* (Dunal) Bitter) and found results that were consistent with ITS-1 sequences, isozymes, and morphology. Mace et al. (1999a) found concordance between AFLPs and other data sets in Datureae, and proposed a new classification for the tribe. AFLPs also have been used in *Solanum tuberosum* to estimate genetic variation and relationships in cultivars (Kim et al. 1998). In potato systematics, Kardolus et al. (1998) used AFLPs in 19 taxa of *Solanum* sect. *Petota* and three taxa of *Solanum* sect. *Lycopersicon*, with results concordant with some current taxonomic hypotheses. Notably, their one placeholder for clade 1 (*S. pinnatisectum*) was basal in the sect. *Petota* clade, concordant with the cpDNA data. McGregor et al. (2002) and Van den Berg et al. (2002) used AFLPs to distinguish morphologically similar species. The objectives of our AFLP study were to use

AFLPs for phylogenetic inference of the North/Central American diploids, to compare the results with the cpDNA and morphological data, with the ultimate goal of using these insights to produce a taxonomic monograph of sect. *Petota* from this region.

Materials and methods

Plant material

Each species was sampled from as many geographically dispersed sites as possible (Table 1). Only one accession was available for *S.* ×*michoacanum*, *S. clarum*, and *S. bulbocastanum* × *S. cardiophyllum* and our conclusions on relationships of these rare species are therefore problematical. The accessions and DNA samples were the same as those used in Lara-Cabrera and Spooner (in press) with minor exceptions. In that prior study, morphological data were not available for *S. morelliforme* because this epiphytic species did not flower, nor did we have living material of the putative interspecific hybrid of *S. bulbocastanum* × *S. cardiophyllum*. We used DNA from two outgroup species, *S. etuberosum* and *S. palustre* (Table 1). Preparations of total DNA were made from fresh leaf tissue from a single individual plant per accession, from plants grown in a greenhouse, following the procedure of Doyle and Doyle (1987), and purified over CsCl/ethidium bromide gradients.

Amplification and DNA fragment analysis

The AFLP™ plant mapping kit (Perkin Elmer Life Sciences, Boston, Massachusetts) was used to produce AFLP fragments, following the manufacturer's directions. Three reactions were performed. The first reaction ligated specific adaptors to the ends of the restriction fragments generated by digestion of DNA with *Eco*R1 and *Mse*1. This was followed by a preselective procedure that amplified fragments with adaptors on both ends by using an *Eco*R1 complementary primer containing a 3' C and an *Mse*1 complementary primer containing a 3' A. The third reaction was a selective amplification, using one of the two primer combinations: primer 1 = *Eco*RI+ ACA/*Mse*I+ CAG, primer 2 = *Eco*RI+ AAC/*Mse*I+ CAC, based on their successful use in the potato study of Kardolus et al. (1998). The primers were fluorescently labeled. The PCR reactions were performed in a 9600 Perkin Elmer thermocycler. PCR products were sent to the Biotechnology Center at the University of Wisconsin-Madison for fluorescent fragment separation. Data were collected using a PE Biosystems DNA Sequencer data collection v. 2.0 and analyzed with GeneScan v. 2.1 (Perkin Elmer Life Sciences). Trace files were later imported into Genotyper (v. 2.1 PE, Perkin Elmer Life Sciences) for fragment sizing. Fragments in the range of 50 to 550 base pairs were included in the analysis, because shorter fragments tended to give less sharp peaks, and longer fragments were not always consistently amplified. Peaks were scored as base-pair sizes and translated into binary presence/absence data. Both primer data sets were combined for analysis. Data are available from the authors.

Cladistic analysis

A cladistic analysis was performed using PAUP 4.0b8 (Swofford 2001). The AFLP data were analyzed using Wagner parsimony (Farris 1970), with *S. etuberosum* and *S. palustre* as outgroups, after the elimination of 15 autapomorphous characters, following Spooner et al. (1993). Trees were produced using a four step search following Olmstead and Palmer (1994): 1) 50,000 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 2 were used as starting trees for a search using NNI for multiple parsimonious trees (MULPARS); 4) trees from step 3 were used as starting trees for a search using TBR and MULPARS with 1000 as the upper limit of trees saved. The resulting trees were used to compute a strict consensus tree. Bootstrap values (Felsenstein 1985) were obtained from 1000 random replicates with NNI, no MULPARS.

Phenetic analysis

Because AFLPs are dominant marker data, and because they represent anonymous fragments, a case has been made that they should be analyzed with phenetic, as well as cladistic methods (e.g. Koopman et al. 2001). AFLP data were analyzed using NTSYS-pc^{R} version 2.02k (Applied Biosystematics, Setauket, New York). The "FIND" option was

enabled to detect all possible trees. Similarity matrices (in SIMQUAL) were generated using the Dice and Jaccard similarity matrix, which ignores shared absent bands. Dice and Jaccard algorithms are appropriate algorithms for predominately dominant AFLP marker data because they give greater weight to the greater information content of dominant presence data. Clustering was performed using the unweighted pair-group method (UPGMA) and Neighbor Joining in SAHN. Cophenetic correlation coefficients were calculated for all combinations of similarity and tree building methods using the procedures COPH and MXCOMP. These coefficients indicate the correlation between a similarity matrix and the phenetic tree resulting from it after a cluster analysis, indicating goodness of fit of the cluster analysis to the similarity matrix. Clustering methods and similarity coefficients are described in Rohlf (1993). Bootstrap values were obtained from the tree with the highest cophenetic coefficient from 5000 random replicates using WinBoot (Yap and Nelson 1996).

Results

Cladistic results

Two primer combinations produced a total of 234 polymorphic bands (no missing data). Wagner parsimony analysis of these bands (Fig. 3a) produced 24 most-parsimonious 1494-step trees with a consistency index (excluding autapomorphies) of 0.157 and a retention index of 0.516. Some internal clades are labeled as A-I for ease of discussion. Clade A contained *S. brachistotrichium*, *S. nayaritense*, *S. stenophyllidium*, and one of the six accessions of *S. cardiophyllum* subsp. *ehrenbergii*. Clade B contained five of the six accessions of *S. cardiophyllum* subsp. *ehrenbergii*, and the sole accession of a putative hybrid of *S. bulbocastanum* × *S. cardiophyllum* subsp. *cardiophyllum* (Rodríguez and Vargas 1994). Clade C contained all accessions of *S. bulbocastanum*, with the two accessions of subsp. *partitum* forming a clade but imbedded in a clade of the other two species that did not form clades. Clade D contained all accessions of *S. trifidum* and *S. tarnii*, and the sole accession of *S. hintonii*. All accessions of *S. trifidum* form a terminal clade but not as a separate clade from *S. tarnii*. Clade E contained all accessions of *S. jamesii*. Clade F contained all accessions of *S. pinnatisectum*, with the two accessions of the putative hybrid *S.* ×*sambucinum* (*S. pinnatisectum* × *S. cardiophyllum* subsp. *ehrenbergii*) at the base of the clade. Clade G contained all accessions of *S. lesteri* and *S. polyadenium*, and the putative hybrid *S.* ×*michoacanum* (*S. bulbocastanum* × *S. pinnatisectum*). Like the *S. tarnii* and *S. trifidum* clade, *S. lesteri* and *S. polyadenium* did not form species-specific clades. Clade H contained all accessions of *S. clarum* and *S. morelliforme*. Clade I contained all accessions of *S. cardiophyllum* subsp. *cardiophyllum*. Clades A, C, D (containing all accessions of *S. trifidum* and *S. tarnii* but excluding *S. hintonii*), E, G (containing all accessions of *S. lesteri* and *S. polyadenium* but excluding *S.* ×*michoacanum*), and clade I are supported with a bootstrap values of $>51\%$ (as shown on the cladogram) and the others were $<50\%$ (not shown on the cladogram).

Phenetic results

The best cophenetic correlation coefficients were produced from the combination of Jaccard/UPGMA (r = 0.904). Successively lower r values were produced by the combination Dice/UPGMA (0.888), Dice/Neighbor Joining (0.822), and Jaccard/Neighbor Joining (0.789). Rohlf (1993) subjectively interprets all values above 0.9 as an excellent fit of the similarity matrix to the resulting tree.

Figure 3b presents the Jaccard/UPGMA phenogram, with dotted lines connecting identical accessions in the cladogram (Fig. 3a). Only a single Jaccard/UPGMA phenogram was found. The cladogram and phenogram outlined almost the same set of species groups, with the major conflict occurring in the arrangement of species within, not among, clades/groups. Exceptions to this concordance of cladistic and phenetic results were: 1) the exclusion in the phenogram from one

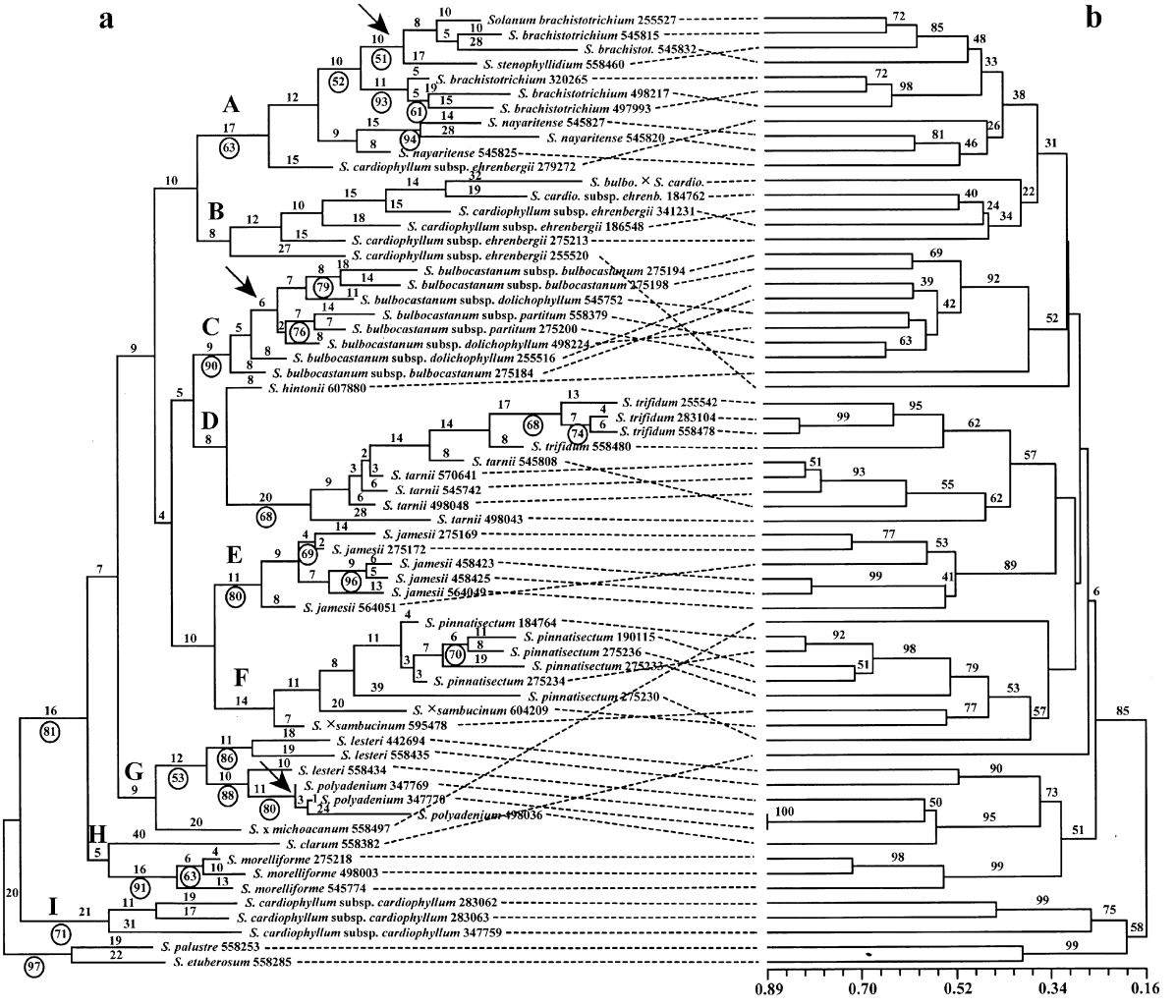

Fig. 3. (a) Cladistic analyses of Amplified Fragment Length Polymorphisms of the North/Central American diploid wild potato (*Solanum* sect. *Petota*) species. The cladogram (left) is one of 24 most-parsimonious 1494-step Wagner trees, drawn as a phylogram, and is rooted by *S. etuberosum* and *S. palustre*, two species in *Solanum* sect. *Etuberosum*. Numbers above the branches represent number of characters supporting each branch. Numbers following the species are USDA Plant Introduction Numbers as listed in Table 1. The three arrows indicate branches that break down in a strict consensus tree. Numbers in circles below the branches represent the bootstrap support values above 50% that were produced in a separate 50% majority rule consensus tree (not shown here). The letters A-I are clades as discussed in the text. **(b)** UPGMA phenogram based on Jaccard coefficient, with overlaid bootstrap values. The dotted lines connect identical accessions in both analyses

accession of *S. cardiophyllum* subsp. *ehrenbergii* to the base of the clade including this taxon, *S. stenophyllidium*, and *S. bulbocastanum*, 2) *Solanum hintonii* grouping in the phenogram with *S. bulbocastanum*, rather than with *S. tarnii* + *S. trifidum*, 3) *Solanum* ×*michoacanum* clustering in the phenogram with *S. pinnatisectum* rather than with *S. lesteri* + *S. polyadenium*, 4) *Solanum clarum* clustering at the base of a large cluster in the phenogram, rather than with *S. morelliforme*, and outside of any particular group, 5) *Solanum pinnatisectum*, *S.* ×*michoacanum*, and *S. jamesii* not clustering in the phenogram as in the cladogram. Conversely, better species-specific clustering is found in the phenogram that placed

S. trifidum and *S. tarnii* in their own groups, rather than the paraphyletic result on the cladogram where *S. trifidum* formed a terminal clade but not as a separate clade from *S. tarnii*.

Discussion

Comparison of AFLP, chloroplast DNA, and morphological results

Our results match those of Koopman et al. (2001) and Mace et al. (1999a,b) who also found that the combination of Jaccard/UPGMA had the similar highest cophenetic correlation coefficients in their AFLP studies. There were many points of agreement in the AFLP, cpDNA (Spooner and Sytsma 1992), and morphological (Lara-Cabrera and Spooner in press) results. In the discussion below, the word "clade" refers to the results of cladistic analysis and "cluster" to the results of phenetic analysis. Despite the use of the same accessions between AFLP and morphological studies, we discuss the dominant binary AFLP data and continuously variable morphological results separately, rather than analyze them as a common dataset. We do this because most wild potato species are very similar and are distinguished morphologically by a series of largely overlapping character states, few if any of which individually are suitable to exclude or to include a taxon from a group (polythetic support) (Spooner et al. 2001, Lara-Cabrera and Spooner in press). The AFLP and morphological data are appropriately analyzed by different similarity algorithms (qualitative classes of algorithms for AFLP, mostly quantitative for morphology). The cpDNA data included the same species as the microsatellite and morphological studies, but generally only one accession per species. However, the few qualitative morphological characters of the North/Central American diploids that could be "mapped" onto the AFLP cladogram are discussed below.

Most clades (Fig. 3a) have low bootstrap support. Except for the 81% cladogram bootstrap support for all North/Central American species exclusive of *S. cardiophyllum* subsp. *cardiophyllum*, (85% bootstrap support on the phenogram, Fig. 3b) none of the external-most branches were well supported. Rather, only more internal branches have bootstrap values that support sister species, species, or accessions within species. Such low external branch support precludes global conclusions of interspecific relationships for all North/Central American species. The interpretation of a bootstrap value as supporting a "true" phylogeny is subject to many factors and it is difficult to choose a value as "significant." Hillis and Bull (1993) showed, in cladistic methods, that under certain conditions thought to be typical of most phylogenetic analyses (equal rates of change, symmetric phylogenies, and internodal changes of equal to or lesser than 20%), bootstrap values of equal to or greater than 70% usually corresponded to a probability of equal to or greater than 90% that a clade was phylogenetically "true." We are not aware of similar studies of bootstrap values for phenetic data. The low consistency index (0.157) and general lack of low bootstrap values on the cladogram indicate much homoplasy that is typical with AFLP data. It also may indicate recent divergence of some North/Central American sister taxa. In cases of recent divergence, strict monophyly in the AFLP results may not yet occur for close sister taxa that are definable morphologically (e.g. *S. tarnii* and *S. trifidum*; *S. lesteri* and *S. polyadenium*).

The phenetic results of the prior morphological study (Lara-Cabrera and Spooner, in press) and AFLP results given here (Fig. 3b) are not designed to show phylogenetic relationships. However, phenetic and cladistic results of the same data can be similar under certain conditions. When similarities due to shared ancestral characteristics or homoplasy exceed similarities due to shared derived characteristics, the phenogram will not represent the phylogeny, but if there were no homoplasy, the phenogram constructed by the phenetic analysis would correspond to the true phylogenetic tree (Futuyma 1998).

***Solanum stenophyllidium, S. brachistotrichium, S. nayaritense, S. cardiophyllum* subsp. ehrenbergii (Clades A, B).** Chloroplast DNA cladistic data (Spooner and Sytsma 1992, Rodríguez and Spooner 1997) and morphological phenetic data (Lara-Cabrera and Spooner in press) supported *S. cardiophyllum* subsp. *ehrenbergii* and *S. nayaritense* as distinct taxa, but suggested that *S. stenophyllidium* and *S. brachistotrichium* were synonyms (*S. stenophyllidium* is the earlier name). Hawkes (1990) distinguished *S. stenophyllidium* by lanceolate leaves vs. *S. brachistotrichium* by linear-lanceolate leaves. Lara-Cabrera and Spooner (in press), however, showed no statistically significant difference of terminal and lateral leaflet shapes between these species.

Clade A also contains one of the six accessions of *S. cardiophyllum* subsp. *ehrenbergii*. *Solanum cardiophyllum* subsp. *ehrenbergii* is morphologically extremely similar to *S. stenophyllidium*, and they are distinguished only by minor differences in leaf shape. This "misplaced accession" of *S. cardiophyllum* subsp. *ehrenbergii* suggests misidentification, but it clustered with other accessions of subsp. *ehrenbergii* in the phenetic study of Lara-Cabrera and Spooner (in press).

***Solanum bulbocastanum, S. cardiophyllum* subsp. *cardiophyllum* (Clades B, I).** *Solanum bulbocastanum* subsp. *bulbocastanum* and subsp. *dolichophyllum* do not form clades. The two accessions of *S. bulbocastanum* subsp. *partitum* form a clade (76% bootstrap support), but this is embedded in the above two subspecies. Previous morphological and nuclear RFLP data (Rodríguez and Spooner 2002) failed to support any of these subspecies, and we think that none of the subspecies of *S. bulbocastanum* are distinct.

Both the AFLP and morphological data (Lara-Cabrera and Spooner in press) widely separated *S. bulbocastanum* (Clade C) from *S. cardiophyllum* subsp. *cardiophyllum* (Clade I), discordant with the cpDNA data that places them in a single clade (Spooner and Sytsma 1992). Supporting the sister taxon relationship of *S. bulbocastanum* and *S. cardiophyllum* subsp. *cardiophyllum* is a morphological synapormorphy of cream-white to light yellow corollas, a color unique to the North/Central American diploids (the other species with corollas pure white or white tinged with blue and purple). The AFLP data, like the cpDNA data, separated *S. cardiophyllum* subsp. *cardiophyllum* from *S. cardiophyllum* subsp. *ehrenbergii*, and allied the latter with *S. brachistotrichium* + *S. stenophyllidium*. The cause of the discordance between different sister taxon relationships of *S. bulbocastanum* and *S. cardiophyllum* subsp. *cardiophyllum* with cpDNA and AFLP data is unknown, but possibly involves a history of "chloroplast capture," a phenomenon well documented in other groups (Wendel and Doyle 1998). We await results from more molecular markers to investigate this discordance.

***Solanum tarnii, S. trifidum, S. hintonii* (Clade D).** AFLP cladistic results place *S. trifidum* and *S. tarnii* in the same clade, but do not separate them into species-specific clades. The AFLP phenetic results, however, place *S. tarnii* and *S. trifidum* into species-specific groups. Their relationship likewise was supported with cpDNA (Spooner and Sytsma 1992) and morphological (Lara-Cabrera and Spooner in press) results. The morphological similarity of these two species is not as evident as there is no clear character to unite them, and *S. tarnii* possesses globose fruits whereas *S. trifidum* possesses conical fruits. The concordance of all data sets, however, suggests that *S. trifidum* and *S. tarnii* are sister taxa. Clade D also contains the sole accession of *S. hintonii*, a species clearly distinguishable from *S. tarnii* and *S. trifidum*.

***Solanum jamesii, S. pinnatisectum,* S. ×*sambucinum* (Clades E, F).** The AFLP results place *S. jamesii*, *S. pinnatisectum*, and the hybrid *S.* ×*sambucinum* in the same clade (<50% bootstrap support). However, all three species are separate in the morphological analysis (Lara-Cabrera and Spooner in press), and the bootstrap support on the AFLP

cladogram is < 50%. *Solanum pinnatisectum* + *S.* ×*sambucinum*, do not cluster with *S. jamesii* on the AFLP phenogram. *Solanum jamesii* and *S. pinnatisectum* are the only two species in sect. *Petota* with pinnatifid stipules, in contrast to the lunate stipules of all other species of wild potato. This striking morphological synapomorphy, and the AFLP cladistic results (despite weak bootstrap support) leads us to suspect that *S. jamesii* and *S. pinnatisectum* are sister taxa; the hybrid nature of *S.* ×*sambucinum* makes statements of cladistic relationship difficult.

***Solanum lesteri, S. polyadenium, S.* ×*michoacanum* (Clade G).** AFLP analyses provide support for the sister taxon relationship of *S. lesteri* and *S. polyadenium*, also concordant with cpDNA (Spooner and Sytsma 1992) and morphological results (Lara-Cabrera and Spooner in press). The phenetic similarity of these two species is clear in the field, as both possess a dense indument of sticky short-stalked glands (Type A trichomes; Gibson 1971), and a characteristic "mousy" odor unique within sect. *Petota*. Both species also possess pentagonal corollas, unique to the North/Central American diploid species (the other species with stellate corollas); *S.* ×*michoacanum* had white stellate corollas.

***Solanum clarum, S. morelliforme* (Clade H).** AFLP data support a sister taxon relationship for *S. clarum* and *S. morelliforme*, but Lara-Cabrera and Spooner (in press) did not examine the latter morphologically because it did not grow well for that study. The cpDNA data (Spooner and Sytsma 1992) also place *S. clarum* and *S. morelliforme* in the same clade, but not into species-specific clades. Hawkes (1990) classified *S. clarum* and *S. bulbocastanum* as the sole two representatives of ser. *Bulbocastana* (Rydb.) Hawkes and placed *S. morelliforme* as the sole representative of ser. *Morelliformia* Hawkes, also with entire leaves. *Solanum morelliforme* and *S. clarum*, however, both grow in moss in epiphytic like conditions, and share many morphological and reproductive traits (discussed in Spooner and Sytsma 1992). Despite low bootstrap support for the clade uniting *S. clarum* and *S. morelliforme* and their failure to cluster in the phenetic results, we consider them as likely sister taxa because of their morphological similarity and AFLP and cpDNA cladistic results.

Hybrid origins

This study included three putative hybrids: 1) *S.* ×*michoacanum* (*S. bulbocastanum* × *S. pinnatisectum*), 2) *S.* ×*sambucinum* (*S. cardiophyllum* subsp. *ehrenbergii* × *S. pinnatisectum*) (Hawkes 1990) 3) an unnamed hybrid *S. bulbocastanum* × *S. cardiophyllum* (Rodríguez et al. 1995). All three of these are distinct from their parental species at least with cladistic results. *Solanum* ×*michoacanum* and *S.* ×*sambucinum* have *S. pinnatisectum* as one of their putative parents, and they always group with this species, but not the other parent. We cannot use additive species-specific AFLP markers to investigate hybrid origins of these three taxa (Rieseberg and Ellstrand 1993) because *S. cardiophyllum* subsp. *ehrenbergii* and S. *pinnatisectum* have no species-specific markers useful to address these questions, and because *S. bulbocastanum* has only two subspecific markers (only in subsp. *partitum*).

Summary

Most of the North/Central American diploid species show some degree of support, both with cladistic and phenetic analyses of AFLP data and phenetic analyses of morphological data. AFLP and morphological data lead us to question the validity of separating *S. brachistotrichium* and *S. stenophyllidium*. In combination with cpDNA and morphological data, AFLP data provide some support for sister taxon relationships for 1) *S. cardiophyllum* subsp. *ehrenbergii* and *S. stenophyllidium* (to include *S. brachistotrichium*), 2) *S. tarnii* and *S. trifidum*, 3) *S. jamesii* and *S. pinnatisectum*, 4) *S. lesteri* and *S. polyadenium*, and 5) *S. clarum* and *S. morelliforme*. Taxonomic decisions on the species boundaries and rela-

tionships of the North/Central American diploid species will be finalized with additional data to result from a much wider examination of herbarium material for our treatment of section *Petota* from North and Central America (Spooner et al. 2004).

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