9

TAXONOMY OF MEXICAN DIPLOID WILD POTATOES (*SOLANUM* SECT. *PETOTA*): MORPHOLOGICAL AND MICROSATELLITE DATA

Sabina I. Lara-Cabrera and David M. Spooner

Abstract. *Solanum* sect. *Petota*, the potato and its wild relatives, contains about 200 wild species distributed from the southwestern United States to central Argentina and adjacent Chile. Most species grow in the Andes, but the United States, Mexico, and Central America contain about 30 diploid (2*n* = 24) taxa, as well as tetraploid (2*n* = 48) and hexaploid (2*n* = 72) ones. Chloroplast DNA restriction site data showed 13 of these 30 taxa to form a clade containing only diploid species, but there was low resolution within the clade. Some of these 13 taxa are similar morphologically, and we questioned whether they were valid species. We analyzed these and related taxa with morphological and microsatellite data. Morphological data showed extensive overlap of putative species-specific characters, but most species could be supported by multivariate techniques, except *S. brachistotrichium* and perhaps *S. stenophyllidium*. Previously mapped nuclear microsatellite markers, developed in *S. tuberosum*, also were explored for help to define species but performed poorly, showing that these microsatellites have reduced phylogenetic utility to analyze the United States, Mexican, and Central American diploid wild potato species.

Key words: microsatellite, potato, simple sequence repeats, *Solanum* sect. *Petota*, SSR.

Solanum sect. *Petota* Dumort., the potato and its wild relatives, contains about 200 wild species distributed from the southwestern United States to central Argentina and adjacent Chile (Hawkes, 1990; Spooner & Hijmans, 2001). Most species grow in the Andes, but the United States, Mexico, and Central America contain about 30 diploid taxa ($2n = 24$), as well as tetraploid ($2n = 48$) and hexaploid ($2n = 72$) ones. The United States, Mexico, and Central American diploids are classified in *Solanum* series *Bulbocastana*, *Morelliformia*, *Pinnatisecta*, *Polyadenia*, and *Tuberosa* (Hawkes, 1990); see Table 1 for authorities of series, species, and subspecies. For simplicity, we refer to species from this region as Mexican or primarily Mexican species. Some Mexican diploids appear very similar morphologically and led us to question whether they were valid species. In this paper we explore the use of morphology and microsatellites (or simple sequence repeats, SSRs) to test the validity of these primarily Mexican diploid species.

Hawkes (1989, 1990) divided *Solanum* sect. *Petota* into 21 series. Two of these series are non-tuber-bearing, and 19 are tuber-bearing. Hawkes (1989, 1990) placed the two non-tuber-bearing series in subsection *Estolonifera* Hawkes, and the remaining 19 tuber-bearing series in subsection *Potatoe* Don of *Solanum*. He divided subsection *Potatoe* into two superseries, *Stellata* Hawkes and *Rotata* Hawkes, based on the corolla shape. Hawkes (1990) and Hawkes and Jackson (1992) proposed a phylogeny of subsection *Potatoe* of *Solanum* based on data from morphology, biogeography, ploidy level, and Endosperm Balance Number. The Endosperm Balance Number (EBN) relates to a strong isolating mechanism in *Solanum* sect. *Petota* and explains success or failure of intra- and interspecific crosses, due to the functioning or breakdown of the endosperm after fertilization. The EBNs are hypothetical genetic factors independent of ploidy and empirically determined, relative to crossing with standard test EBN crossers of known EBN. In potato, these are 2x(1EBN), 2x(2EBN), 4x(2EBN), 4x(4EBN), and 6x(4EBN). Species can cross within EBN, irrespective of ploidy (Hanneman, 1994).

Hawkes (1990) and Hawkes and Jackson (1992) informally designated subsets of *Solanum* species within each superseries with more "primitive" or "advanced" morphological characters, providing four groups, primitive *Stellata*, advanced *Stellata*, primitive *Rotata*, and advanced *Rotata*. In this theory the Mexican diploids are primitive *Stellata* (in ser. *Bulbocastana*, *Morelliformia*, *Pinnatisecta*, and *Polyadenia*). All *Solanum* species in these series are 2x(1EBN). Representatives of these primitive *Stellata* in *Solanum* migrated to South America and remained primitive *Stellata* (ser. *Circaeifolia*, *Commersoniana*, *Lignicaulia*, *Olmosiana*). These then evolved to advanced *Stellata*, then to primitive *Rotata*, and finally to advanced *Rotata*. The species in Mexico that are advanced *Rotata* (*S. verrucosum* of ser. *Tuberosa*, and members of ser. *Conicibaccata*, *Demissa*, and *Longipedicellata*) either remigrated back from South America, or are products of hybridization between these remigrants and primitive indigenous Mexican diploids. For the sake of simplicity, all *Solanum* species of primitive *Stellata* in the United States, Mexico, and Guatemala will here be referred to as "primitive Mexican diploids."

Spooner et al. (1991) investigated the Hawkes (1990) *Stellata–Rotata* hypothesis using chloroplast DNA (cpDNA) restriction site analysis in *Solanum* series *Bulbocastana*, *Conicibaccata*, *Demissa*, *Longipedicellata*, *Morelliformia*, *Pinnatisecta*, *Polyadenia*, and *Tuberosa*. Their results gave partial support to the Hawkes (1989, 1990) and Hawkes and Jackson (1992) hypotheses, by supporting most primitive Mexican diploids as basal, and other Mexican species as advanced. Some putative primitive *Stellata* from South America, however, such as *Solanum circaeifolium* (ser. *Circaeifolia*), were later supported as derived by further cpDNA restriction site studies (Spooner & Sytsma, 1992; Spooner & Castillo, 1997).

Two other cpDNA restriction site studies investigated relationships of Mexican potatoes. Spooner and Sytsma (1992) produced a clado-

TABLE 1.

Location, series affiliation within *Solanum*, PI number, and collector, of the accessions examined in this study. Herbarium vouchers are deposited at PTIS, along with other known herbaria of deposition in the locality column (CIP = International Potato Center in Lima, Peru, INIFAP = the Mexican National Potato Program in Toluca, Mexico). [1]Accession number corresponds to Figs. 2, 4. [2]The map locality corresponds to Fig. 1. SA refers to South America and U refers to localities with unknown location beyond the country of origin. [3]United States Department of Agriculture Plant Introduction Numbers.

Accession Number[1]	Map Locality[2]	Series placement by Hawkes (1990)	Taxon	PI[3]	Collector	Locality
1	21	*Bulbocastana* (Rydb.) Hawkes	*Solanum bulbocastanum* Dunal subsp. *bulbocastanum*	275198	*Hawkes 1595*	MEXICO. **México**: Cerro de Cocotitlán (IBUG, MEXU, PTIS)
2	21		*S. bulbocastanum* subsp. *bulbocastanum*	275184	*Hawkes 1581*	MEXICO. **Federal District**: Pedregal de San Ángel, Ciudad Universitaria (IBUG, IEB, MEXU, PTIS, WIS)
3	23		*S. bulbocastanum* subsp. *bulbocastanum*	275194	*Hawkes 1591*	MEXICO. **Oaxaca**: ruins of Montealbán (IBUG, PTIS)
4	14		*S. bulbocastanum* subsp. *dolichophyllum* (Bitter) Hawkes	498224	*Ochoa 14162*	MEXICO. **Michoacán**: Las Palmitas (CIP, IBUG, K, PTIS, WIS)
5	21		*S. bulbocastanum* subsp. *dolichophyllum*	545752	*Tarn et al. 244*	MEXICO. **México**: E of Coatepéc Harinas, between San Francisco and Porfirio Díaz (B, K, IBUG, PTIS)
6	17		*S. bulbocastanum* subsp. *dolichophyllum*	255516	*Graham 300B*	MEXICO. **Jalisco**: Cuidad Guzmán (PTIS)
7	28		*S. bulbocastanum* subsp. *partitum* (Correll) Hawkes	558379	*Spooner et al. 4224*	MEXICO. **Chiapas**: 9.4 km S of Rt. 190 (from near Teopisca), to Las Rosas (IBUG, INIFAP, PTIS, WIS)
8	29		*S. bulbocastanum* subsp. *partitum*	275200	*Hawkes 1796*	GUATEMALA. **Huehuetenango**: rd. from Huehuetenango to Quezaltenango, 5 km S of Manlacatancito (C, K, PTIS)

TABLE 1 CONTINUED.

Accession Number[1]	Map Locality[2]	Series placement by Hawkes (1990)	Taxon	PI[3]	Collector	Locality
9	27		*S. clarum* Correll	558382	*Spooner et al. 4216*	MEXICO. **Chiapas**: 1.1 km N of town square of El Porvenir on rd. to Siltepecguez (IBUG, INIFAP, PTIS)
10	23	*Morelliformia* Hawkes	*S. morelliforme* Bitter & Münch	275220	*Hawkes 1648*	MEXICO. **Veracruz**: Km 305.5 from México on the rd. from Las Vigas to Jalapa, Malpaís de La Joya (C, K, P, PTIS)
11	16		*S. morelliforme*	275218	*Hawkes 1613*	MEXICO. **México**: 44.5 km from the main Toluca–Morelia Rd., towards Valle de Bravo from Toluca (C, PTIS, US)
12	16		*S. morelliforme*	498003	*Tarn et al. 48*	MEXICO. **México**: about 18.5 km towards Valle de Bravo from Toluca–Temascaltepec, Hwy. 134 via Rancho San Ramón (PTIS)
13	5	*Pinnatisecta* (Rydb.) Hawkes	*S. brachistotrichium* (Bitter) Rydb.	320265	*Hawkes 1234*	MEXICO. **Chihuahua**: Majalca, 40 mi. NW of Chihuahua, near the village (C, IBUG, PTIS, US)
14	5		*S. brachistotrichium*	498217	*Ochoa 14206*	MEXICO. **Chihuahua**: Rt. Chihuahua–Ciudad Juárez (CIP, IBUG, PTIS, US, WIS)
15	6		*S. brachistotrichium*	497993	*Tarn et al. 13*	MEXICO. **Chihuahua**: La Aurora at 41 km, along rd. to San Juanito from the La Junta to Yepachic Hwy. (PTIS)
16	8		*S. brachistotrichium*	545832	*Tarn et al. 207*	MEXICO. **Aguascalientes**: from Rincón de Ramos on Hwy. 45, 30 km along the track towards El Chiquihuitillo, Rancho Tierra Colorada (BR, C, K, MEXU, PTIS)

Accession Number[1]	Map Locality[2]	Series placement by Hawkes (1990)	Taxon	PI[3]	Collector	Locality
17	8		*S. brachistotrichium*	545815	*Tarn et al. 215*	MEXICO. **Aguascalientes**: Hwy. 70, about 33 km from Aguascalientes, 8.2 km along the track past Milpillas de Arriba towards Potrero (PTIS)
18	8		*S. brachistotrichium*	255527	*Graham 346 X 348*	MEXICO. **Aguascalientes**: between Aguascalientes and Calvillo (PTIS)
19	22		*S. cardiophyllum* Lindl. subsp. *cardiophyllum*	347759	*Tarn 241D*	MEXICO. **Puebla**: left side of Tehuacán–Huajuapan de León rd. on the Puebla side of border, Hwy. 125, almost at border of Puebla–Oaxaca states (PTIS)
20	U		*S. cardiophyllum* subsp. *cardiophyllum*	283062	*Graham s.n.*	MEXICO (PTIS)
21	U		*S. cardiophyllum* subsp. *cardiophyllum*	283063	*Graham s.n.*	MEXICO (PTIS)
22	11		*S. cardiophyllum* subsp. *ehrenbergii* Bitter	279272	*Hawkes 1458*	MEXICO. **Aguascalientes**: 7 mi. from Aguascalientes on the rd. to Loreto, 1.25 mi. from the main Aguascalientes to Zacatecas Hwy. (C, K, PTIS, US)
23	12		*S. cardiophyllum* subsp. *ehrenbergii*	184762	*Hawkes 1086*	MEXICO. **Querétaro**: San Juan del Río, SW of the town (ECON, PTIS)
24	8		*S. cardiophyllum* subsp. *ehrenbergii*	341231	*Commonwealth Potato Collection 2302*	MEXICO. **Jalisco**: Km 201 from Guadalajara on the rd. to San Luis Potosí (PTIS)
25	12		*S. cardiophyllum* subsp. *ehrenbergii*	186548	*Hawkes 1100*	MEXICO. **Zacatecas:** 726.5 km from México towards Zacatecas (LL, PTIS)
26	9		*S. cardiophyllum* subsp. *ehrenbergii*	255520	*Graham 371*	MEXICO. **San Luis Potosí** (PTIS)
27	12		*S. cardiophyllum* subsp. *ehrenbergii*	275213	*Hawkes 1428*	MEXICO. **Querétaro**: 3 km SSE of town (C, K, PTIS, US)

TABLE 1 CONTINUED.

Accession Number[1]	Map Locality[2]	Series placement by Hawkes (1990)	Taxon	PI[3]	Collector	Locality
28	22		*S. hintonii* Correll		*Spooner et al. 4033*	MEXICO. **México:** 6.5 km SW of Temascaltepec–Valle de Bravo rd., on rd. to San Pedro Tenayac, on S side of rd., ca. 50 m downstream of bridge over rd. (INIFAP, PTIS)
29	2		*S. jamesii* Torr.	458425	*Ugent & Ruhde 16-78*	U.S.A. **Arizona**: Apache Co., Hwy. 180, 3.2 km S of Eager at the junction with Rt. 130 (WIS, PTIS)
30	2		*S. jamesii*	564051	*Salas et al. 24*	U.S.A. **Arizona**: Apache Co., near Nelson reservoir, two mi. S on Rt. 666 then E 1 mi. on rd. 275 (PTIS)
31	3		*S. jamesii*	564049	*Salas et al. 21*	U.S.A. **New Mexico**: Catron Co., Reserve vicinity, at about 6 mi. NE of Aragon on Hwy. 12, at 33 mi. marker (from Hwy. 180) (PTIS)
32	1		*S. jamesii*	275169	*Hawkes 1176*	U.S.A. **New Mexico**: Grant Co., S end of the Big Burro Mountains, Silver City, 17 mi. from Lordsburg (Rt. 180) (K, PTIS)
33	1		*S. jamesii*	458423	*Ugent & Ruhde 7-78*	U.S.A. **New Mexico**: Grant Co., Hwy. 90, 43.5 km SW of Silver City, Gila National Forest (PTIS, WIS)
34	2		*S. jamesii*	275172	*Hawkes 1207*	U.S.A. **Arizona**: Cochise Co., Chiricahua Mountains, Monita Canon, just below Faraway Ranch at the edge of National Monument (K, PTIS)

Accession Number[1]	Map Locality[2]	Series placement by Hawkes (1990)	Taxon	PI[3]	Collector	Locality
35	20		*S.* ×*michoacanum* (Bitter) Rydb. (putative origin is *S. bulbocastanum* × *S. pinnatisectum*)	558497	*Spooner et al. 4077*	MEXICO. **Michoacán**: at km 21 marker along Rt. 120 S of Morelia, on W side of Rt. 120 (INIFAP, PTIS)
36	7		*S. nayaritense* (Bitter) Rydb.	545827	*Tarn et al. 270A*	MEXICO. **Nayarit**: near Sta. Anita, about 1.5 km NE of Sta. Teresa (PTIS)
37	8		*S. nayaritense*	545820	*Tarn et al. 234A*	MEXICO. **Zacatecas**: Hwy. 54, 10 km SW of Jalpa, 26.3 km along the track toward Tlaltenango (K, PTIS)
38	8		*S. nayaritense*	545825	*Tarn et al. 225*	MEXICO. **Zacatecas**: Hwy. 70, 12 km NW of Jalpa, 18 km along the track SE toward Tlachichila (K, PTIS)
39	13		*S. pinnatisectum* Dunal	275234	*Hawkes 1456*	MEXICO. **Jalisco**: 15 mi. from León on the rd. to Aguascalientes (C, IBUG, K, MEXU, PTIS, US)
40	11		*S. pinnatisectum*	184764	*Hawkes 1093*	MEXICO. **Guanajuato**: León, 2 km SE of the cement factory (BR, IBUG, K, LL, MEXU, MPU, NY, PTIS, WAG)
41	14		*S. pinnatisectum*	275233	*Hawkes 1455*	MEXICO. **Guanajuato**: 2 mi. from Siláo on the rd. to Guanajuato, El Castillo (C, IBUG, K, MEXU, PTIS)
42	13		*S. pinnatisectum*	275236	*Hawkes 1505*	MEXICO. **Jalisco**: 29 mi. from Guadalajara on the rd. to Mexico City, hacienda de Huejotitan (C, IBUG, K, MEXU, PTIS, US)
43	12		*S. pinnatisectum*	275230	*Hawkes 1424*	MEXICO. **Querétaro**: 3 km SSE of town, by the small reservoir (IBUG, K, MEXU, PTIS)
44	20		*S. pinnatisectum*	190115	*Baldwin 14374*	MEXICO. **Michoacán**: Morelia (LL, NA, PTIS)
45	13		*S. bulbocastanum* × *S. cardiophyllum* putative hybrid		*Rodríguez 2592*	MEXICO. **Jalisco**: Sierra de La Primavera (PTIS)

TABLE 1 CONTINUED.

Accession Number[1]	Map Locality[2]	Series placement by Hawkes (1990)	Taxon	PI[3]	Collector	Locality
46	11		*S.* ×*sambucinum* Rydb. (putative origin is *S. ehrenbergii* × *S. pinnatisectum*)	595478	*Rodríguez 2563*	MEXICO. **Querétaro**: foot-hills of "El Buey" mountain, on junction of roads of Querétaro–San Luis Potosí and Mexico City–San Luis Potosí (F, IBUG, MICH, MO, NY, PTIS)
47	18		*S.* ×*sambucinum*	604209	*Rodríguez 2565*	MEXICO. **Guanajuato**: La Purísima, municipality of San Diego de la Unión, rd. from Querétaro city to San Luis Potosí (MICH, PTIS)
48	10		*S. stenophyllidium* Bitter	558460	*Spooner et al. 4104*	MEXICO. **Jalisco**: rd. from N side of Guadalajara, past side rd. to San Francisco Tesistán to San Cristóbal de la Barranca, on W side of rd., 20.5 km (by posted km signs), N of Guadalajara, about 0.5 km S of Mirador (INIFAP, PTIS)
49	16		*S. tarnii* Hawkes & Hjert.	498048	*Tarn et al. 79*	MEXICO. **Hidalgo**: from Las Trancas, Hwy. 85, Zimapan to Jacala (K, PTIS)
50	16		*S. tarnii*	545808	*Tarn et al. 257*	MEXICO. **Hidalgo**: turning off Hwy. 85 (Zimapan/Jacala) at Las Trancas, go 6.4 km along rd. towards Nicolás Flores (C, F, K, MEXU, PTIS, WAG)
51	15		*S. tarnii*	545742	*Tarn et al. 56*	MEXICO. **Veracruz**: about 14 km S of Huayacocotla, near Viborillos, on rd. to Tulancingo (K, PTIS)
52	16		*S. tarnii*	498043	*Tarn et al. 57*	MEXICO. **Hidalgo**: 28 km S of Huayacocotla, 8 km before Agua Blanca (C, K, MEXU, PTIS)

Accession Number[1]	Map Locality[2]	Series placement by Hawkes (1990)	Taxon	PI[3]	Collector	Locality
53	16		*S. tarnii*	570641	*Hjerting 7361*	MEXICO. **Hidalgo**: 20 km from Agua Blanca towards Huayacocotla, near Palo Bendito (PTIS)
54	17		*S. trifidum* Correll	255542	*Canada Ottawa 460*	MEXICO. **Michoacán**: Km 48 on the Carapan–Uruapan Hwy. (PTIS)
55	18		*S. trifidum*	558478	*Spooner et al. 4283*	MEXICO. **Michoacán**: on rd. from Rt. 120 NW to Cheran, 2.5 km SE of Serina (INIFAP, PTIS, WIS)
56	17		*S. trifidum*	558480	*Spooner et al. 4089B*	MEXICO. **Michoacán**: along microwave tower rd. off of dirt rd. beginning about 5 km NE of Capacuaro, 5.9 km from beginning of this microwave tower rd. (INIFAP, PTIS)
57	17		*S. trifidum*	283104	*Hawkes 1541*	MEXICO. **Jalisco**: NE slope of Nevado de Colima, 13 km from turn to Tolimán on the rd. from Ciudad Guzmán, Los Alpes (IBUG, K, MEXU, PTIS)
58	U		*S. trifidum*	292213	*Ugent 5745*	MEXICO (PTIS)
59	26	*Polyadenia* Bukasov	*S. lesteri* Hawkes & Hjert.	558434	*Spooner et al. 4155*	MEXICO. **Oaxaca**: on W side of Rt. 125, 3.4 km S of Rt. 190, just S of San Juan Teposcolula (INIFAP, PTIS, WIS)
60	26		*S. lesteri*	442694	*Hawkes et al. 1714*	MEXICO. **Oaxaca**: 25.8 km S of Miahuatlán on the Oaxaca–Puerto Ángel rd. (K, PTIS)
61	26		*S. lesteri*	558435	*Spooner et al. 4177*	MEXICO. **Oaxaca**: along E side of Rt. 175, at km 134.1, about 50 m off rd.; up a slope, S of Miahuatlán de Porfirio Díaz (INIFAP, PTIS, WIS)

TABLE 1 CONTINUED.

Accession Number[1]	Map Locality[2]	Series placement by Hawkes (1990)	Taxon	PI[3]	Collector	Locality
62	22		*S. polyadenium* Greenm.	498036	*Tarn et al. 65*	MEXICO. **Hidalgo**: Hwy. 85, Zimapan to Tamzunchale, at Las Trancas, about 8 km E at Jaguey on the track to Nicolás Flores (PTIS)
63	13		*S. polyadenium*	558450	*Spooner et al. 4137*	MEXICO. **Jalisco**: on microwave tower rd. to Cerro Grande, SE of Sante Fé, 1.1 km downhill of top of town (INIFAP, PTIS, WIS)
64	24		*S. polyadenium*	161728	*Correll 14374*	MEXICO. **Michoacán**: near Matugeo (ECON, GAT, MEXU, PTIS)
65	25		*S. polyadenium*	347769	*Tarn & Gómez 221*	MEXICO. **Puebla**: New México–Veracruz, toll rd., at km 208.5 (K, PTIS)
66	19		*S. polyadenium*	347770	*Tarn & Gómez 234*	MEXICO. **Veracruz**: near Puerto del Aire, on old Orizaba–México rd., Rt. 150, between Puerto and Veracruz–Puebla state boundary (K, PTIS)
67	SA	*Circaeifolia* Hawkes	*S. circaeifolium* Bitter	498188	*Hawkes et al. 6581*	BOLIVIA. **Cochabamba**: Ayopaya, near Independencia, Ichupampa (PTIS)
68	SA	*Commersonia* Bukasov	*S. commersoni* Dunal	566751	*Hoffman s.n.*	ARGENTINA (PTIS)
69	SA	*Conicibaccata* Bitter	*S. santollalae* Vargas	473372	*Hawkes et al. 5103*	PERU. **Cuzco**: Paucartambo, Pillahuata (PTIS)
70	SA	*Lignicaulia* Hawkes	*S. lignicaule* Vargas	275273	*E. Baur Sortiment 1884*	PERU. **Cuzco**: Calca, Pisac (PTIS)
71	2	*Longipedicellata* Bukasov	*S. fendleri* subsp. *fendleri* Hawkes	275163	*Hawkes 1180*	U.S.A. **Arizona**: Cochise Co., Chiricahua Mountains, Rustler Park (K, PTIS)
72	16	*Tuberosa* (Rydb.) Hawkes	*S. verrucosum* Schltdl.	275260	*Hawkes 1658*	MEXICO. **Hidalgo** (AA, BM, C, K, PTIS)

gram that divided the primitive Mexican diploid species into these clades in *Solanum*: (1) series *Pinnatisecta*, with internested series *Polyadenia*, *Bulbocastana* (in part), and series *Morelliformia*; (2) *S. bulbocastanum* and *S. cardiophyllum*; and (3) *S. verrucosum* and all Mexican polyploid species. The second clade was unexpected because all prior hypotheses suggested that *S. bulbocastanum* and *S. cardiophyllum* would associate with the first clade. *Solanum verrucosum*, on the other hand, was long recognized by morphological and crossing data to truly be distinct and to belong in the third clade. Rodríguez and Spooner (1997) reanalyzed the *Solanum bulbocastanum* and *S. cardiophyllum* clade including more accessions and all the subspecies of each species. The resulting tree maintained all the subspecies in the same clade except for all the accessions of *S. cardiophyllum* subsp. *ehrenbergii*. This subspecies was placed in the basal Mexican diploid clade, most closely related to *S. brachistotrichium* and *S. stenophyllidium*.

The basal relationship of the primitive *Stellata* within *Solanum* sect. *Petota,* including *S. circaeifolium* of series *Circaeifolia,* was further investigated by Kardolus (1998) using amplified fragment length polymorphisms (AFLP). *Solanum circaeifolium* was shown to be primitive by AFLPs and nested with the Mexican diploids, unlike cpDNA results (Spooner & Castillo, 1997), suggesting a "chloroplast capture" event, known to be common throughout angiosperms (Rieseberg & Brunsfeld, 1992). Its primitive position, however, matched the *Stellata/Rotata* hypothesis of Hawkes (1989, 1990) and Hawkes and Jackson (1992).

Microsatellites are hypervariable molecular markers (Tautz, 1989; Powell et al., 1996), consisting of tandem repeats of 1 to 6 bp (Provan et al., 1996b; Rubinsztein et al., 1999; Bichara et al., 2000). They have been used in cultivar identification (Smulders et al., 1997; Lin & Walker, 1998), germplasm analysis (Powell et al., 1996), the development of quantitative trait loci (QTL) (Arens et al., 1995; Areshchenkova & Ganal, 1999; Winter et al., 1999; Lorieux et al., 2000), to detect genetic polymorphisms (Russell et al., 1997; Nader et al., 1999; Teulat et al., 2000), and in phylogenetic studies (Kostia et al., 2000; Petren et al., 1999; Clisson et al., 2000; Raker & Spooner, 2002). In potato they have been used mainly for cultivar identification (Milbourne et al., 1997; Schneider & Douches, 1997), to develop linkage groups (Milbourne et al., 1998), in germplasm identification (Provan et al., 1996b), in analysis of intra-specific somatic hybrids (Provan et al., 1996a), and in genetic analysis of anther-derived haploid potatoes (Veilleux et al., 1995).

Milbourne et al. (1998) developed microsatellite primers and mapped them to all 12 linkage groups of cultivated potato, *S. tuberosum*. We wished to test their use to investigate the validity and interspecific relationships of the primitive Mexican diploids and other primitive and advanced potatoes, including members of Hawkes's (1990) primitive *Stellata*. Raker and Spooner (2002) showed these primers to be of use to distinguish the subspecies of *S. tuberosum*, but this was the very germplasm base from which these primers were developed. We needed these data for our ongoing floristic studies of *Solanum* sect. *Petota* in North America, Mexico, and Central America. This included investigations of hybrid origins for some of these species. These hybrids and putative origins by Hawkes (1990) are *S.* ×*michoacanum* (*S. bulbocastanum* × *S. pinnatisectum*), and *S.* ×*sambucinum* (*S. cardiophyllum* subsp. *ehrenbergii* × *S. pinnatisectum*). In addition, Rodríguez and Vargas-P. (1994) postulated a new unnamed hybrid of *S. bulbocastanum* × *S. cardiophyllum* (no subspecies designated for either species). We include South American primitive *Stellata* species to test the Hawkes (1989, 1990) and Jackson and Hawkes (1992) hypotheses of relationships in *Solanum*.

Materials and Methods

PLANT MATERIAL

More than one accession per taxon was analyzed (when available) from as many geographically dispersed sites as possible (Table 1, Fig. 1). The plants were grown in the greenhouse, because many of the primitive Mexican diploids do not thrive outdoors in Wisconsin. An average of three plants per accession of the Mexican diploids were grown, and one accession per series from the South American diploids. *Solanum stenophyllidium* had only one accession available as seeds, and we analyzed two separate sets of three plants per the same accession for morphological analysis.

MORPHOLOGY AND DATA ANALYSIS

Morphological characters were measured when the plants were in full flower (Table 2). Significant differences in character states for quantitative characters were studied by the Tukey-Kramer test, and qualitative characters by the Likelihood Ratio Chi square test and Pearson Chi square test in JMP statistical software v. 3.1.5 (SAS Institute Inc., 1998). Phenetic analyses were performed with NTSYS-pc (Rohlf, 1992). An operational taxonomic unit (OTU) was the average of three individuals per accession. Data were standardized (STAND), and similarity matrices (in SIMINT), average taxonomic distance (DIST), Euclidean distance (EUCLID), Manhattan distance (MANHAT), and product-moment correlation (CORR) were generated. Clustering was performed with the unweighted pair-group method, UPGMA (Sokal & Sneath, 1963), and Neighbor Joining, NJ (Saitou & Nei, 1987). Cophenetic correlation coefficients (COPH, in MXCOMP) were used to measure the distortion between similarity matrices and the resultant four phenograms. Stepwise discriminate analyses (SDA) were performed by SAS v.7 (SAS Institute Inc., 1998) using Stepwise discriminate analysis (STEPDISC).

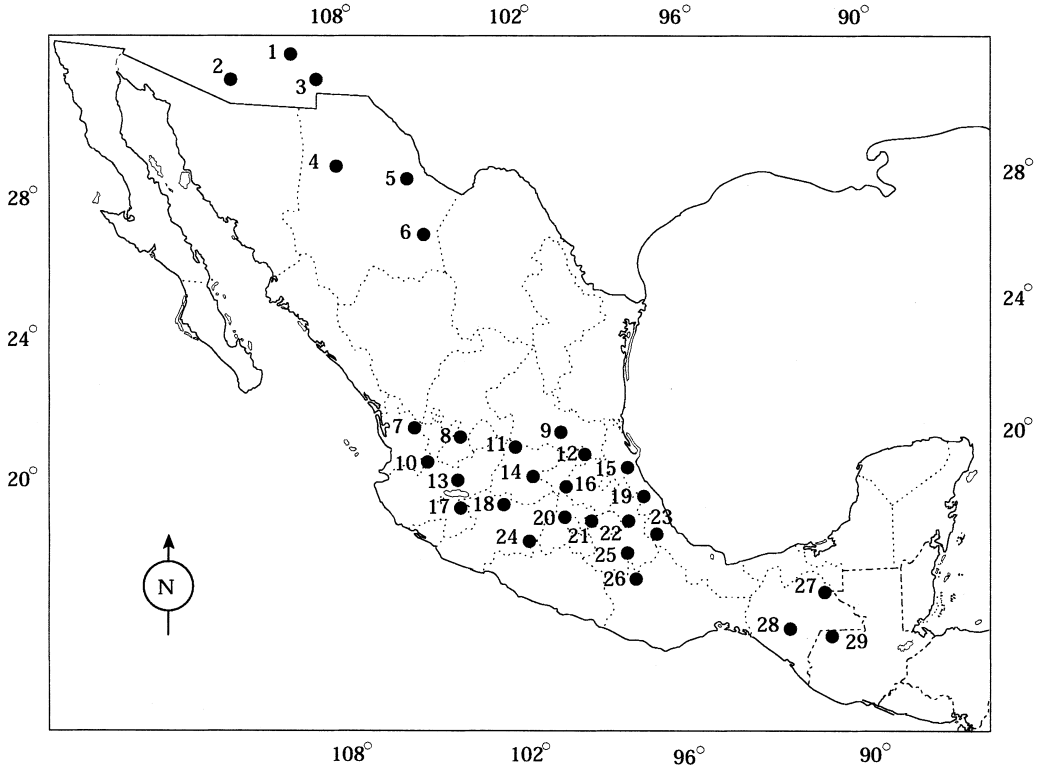

Figure 1. Distribution of wild potato accessions used in this study from the United States, Mexico, and Guatemala. Accessions from South America are not mapped. Numbers correspond to generalized map localities in Table 1.

Table 2.

Morphological characters measured. All values are in cm, unless otherwise indicated. The 35 of 56 statistically significant characters ($P = 0.05$, SAS Institute Inc., 1998) distinguishing all taxa (see text) are marked with an asterisk (*).

STEM CHARACTERS

1. Stem pubescence length (mm)*. 2. Stem pubescence posture: pointing up (1), pointing down (2), pointing out (3)*. 3. Stem diameter (mm)*. 4. Stem wings: present (1), absent (2). 5. Stem shape: angular (1), rounded (2)*. 6. Pubescence type: glabrescent (1), pubescent (2)*.

LEAF CHARACTERS

7. Leaf width*. 8. Ratio: leaf length/width*. 9. Number of leaflet pairs. 10. Leaflet position: alternate (1), opposite (2). 11. Widest leaflet pair: terminal (1), most distal lateral leaflet pair (2), second most distal lateral leaflet pair (3)*. 12. Interstitial leaflets: present (1), absent (2)*. 13. Terminal leaflet width*. 14. Ratio: length/width of terminal leaflet blade*. 15. Length from widest point of terminal leaflet blade to base of blade*. 16. Ratio: length from widest point of terminal leaflet blade to base of blade/length of terminal leaflet blade. 17. Petiolule length of terminal leaflet*. 18. Most distal lateral leaflet width*. 19. Ratio: length of most distal lateral leaflet blade/width of most distal lateral leaflet*. 20. Length from widest point of most distal lateral leaflet blade to base of blade*. 21. Ratio: length from widest point of most distal lateral leaflet blade to base of blade/length of most distal lateral leaflet blade*. 22. Petiolule length of most distal lateral leaflet*. 23. Second most distal lateral leaflet width*. 24. Ratio: length of second most distal lateral leaflet blade/width of second most distal lateral leaflet*. 25. Length from widest point of second most distal lateral leaflet blade to base of blade*. 26. Ratio: length from widest point of second most distal lateral leaflet blade to base of blade/length of second most distal lateral leaflet blade. 27. Petiolule length of second most distal lateral leaflet*. 28. Abaxial leaf pubescence length (mm)*. 29. Abaxial leaf pubescence posture: pointing up (1), pointing down (2), pointing out (3). 30. Lateral leaflet decurrency: decurrent (1), not decurrent (2)*. 31. Petiole wings: present (1), absent (2)*. 32. Petiole length*. 33. Shape of pseudostipular leaflet: pinnulate (1), lunate (2)*. 34. Spicy odor: present (1), absent (2)*.

FLORAL CHARACTERS (see Spooner & van den Berg, 1992, for illustrations of characters 49–52).

35. Peduncle length. 36. Length from the base to the articulation of the pedicel*. 37. Ratio: pedicel length/length from base to the articulation of the pedicel. 38. Length of calyx (mm). 39. Ratio: length of calyx lobe/length of calyx*. 40. Posture of calyx lobe: straight (1), bent over (2), curled around (3). 41. Symmetry of calyx: symmetrical (1), asymmetrical (2). 42. Length of calyx acumen (mm)*. 43. Ratio: length of calyx lobe/length of calyx acumen (mm). 44. Calyx color: green (1), purple (2), green and purple (3). 45. Position of calyx and corolla: alternate (1), opposite (2). 46. Style exsertion (mm). 47. Length of anther (mm). 48. Length of anther filament (mm). 49. Radius of the corolla measured from the center of the corolla to the tip of the corolla lobes (mm). 50. Ratio: Radius of the corolla measured from the center of the corolla to the tip of the corolla lobes/distance between the center of the flower and the junction of the corolla lobes (mm)*. 51. Width of the corolla lobe measured at the base of the corolla junction (mm)*. 52. Ratio: width of the corolla lobe measured at the base of the corolla junction/length from a line drawn across widest point of corolla lobes (mm). 53. Primary color of the abaxial surface of the corolla: white (1), creamy white (2), purple (3)*. 54. Secondary color of the abaxial surface of the corolla: white (1), creamy white (2), purple (3). 55. Posture of style: erect (1), curved (2).

FRUIT CHARACTERS

56. Fruit shape: globose (1), conical (2)*.

We used stepwise discriminant analysis to discover characters that best distinguished the nine most phenetically similar primitive Mexican diploid species (*Solanum brachistotrichum*, *S. cardiophyllum* subsp. *ehrenbergii*, *S. hintonii*, *S. jamesii*, *S.* ×*michoacanum*, *S. nayaritense*, *S. stenophyllidium*, *S. trifidum*, *S. tarnii*), with all procedures as above.

DNA EXTRACTION, AMPLIFICATION, AND DNA FRAGMENT ANALYSIS

DNA was extracted using the protocol in Raker and Spooner (2002). Forty-five mapped primers were selected from Milbourne et al. (1998) that were designed for *S. tuberosum* and screened for ability to amplify a fragment in these species: stm0001, stm0003, stm0007, stm0010, stm0013, stm0014, stm0017, stm0019, stm0024, stm0025, stm0028, stm0032, stm0037, stm0038, stm0051, stm0052, stm1003, stm1005, stm1008, stm1017, stm1020, stm1021, stm1024, stm1025, stm1029, stm1031, stm1041, stm1049, stm1055, stm1058, stm1064, stm1069, stm1100, stm1102, stm1104, stm1106, stm2005, stm2012, stm2013, stm2020, stm2022, stm2028, stm3009, stm3010, stm3016. Forward primers were labeled with fluorescent dyes FAM (blue), HEX (yellow), and TETRA (green), from PE Biosystems, to amplify individual accessions of all of the species. Reaction conditions were optimized, modifying conditions listed in Provan et al. (1996b). Conditions for a 25 µl reaction included 1X PCR Buffer II (Perkin Elmer), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM of each primer pair, 1 unit of AmpliTaq Gold® (Perkin Elmer), and 10–20 ng of DNA. The annealing temperature followed Milbourne et al. (1998) or 1ºC to 2ºC below (Table 3). All reactions were amplified in a 9600 PE thermocycler with the following times and temperatures: 1 cycle for 10 min. at 94°C, 2 min. to anneal temperature, 5 min. at 72°C, 29 cycles of 1 min. at 94°C, 45 sec. to anneal, 5 min. at 72°C, ending with a 72°C hold for 45 min.

The microsatellite products for each individual were pooled in dye combinations of 3 µl FAM: 6 µl TETRA: 9 µl HEX, or in some cases each product was analyzed separately. PCR products were sent to the Biotechnology Center at the University of Wisconsin-Madison for fragment separation. Products were processed as follows: 1.5 µl aliquots of PCR product were mixed with 1.8 µl sample loading buffer (80% formamide, 10 mg/ml dextran blue, 5 mM EDTA at pH 8.0) and 0.45 µl of TAMRA 500 molecular weight standard (PE Biosystems). The mixture was heated for 3 min. at 95°C and then placed on ice. Approximately 1 µl of the cold samples was loaded on a 5% LongRanger™ (FMC Bioproducts) polyacrylamide/6M urea gel in a PE Biosystems 377XL DNA sequencing machine. Samples were run at 3000 V with 2400 scans/hour in 36 cm well-to-read plates. Data were collected using a PE Biosystems DNA Sequencer data collection v. 2.0 and analyzed with GeneScan v. 2.1 (PE Biosystems). Data were later imported in to Genotyper v. 2.1 PE Applied Biosystems for allele sizing, both automatically and manually. Peaks were scored as allele sizes in bp and manually translated into binary presence/absence.

MICROSATELLITE DATA ANALYSIS

The multistate matrix was imported to Microsat v. 1.5d (Minch et al., 1997) to produce distance matrices with two models, absolute difference and delta mu squared $(\partial\mu)^2$, both of which assume a step-wise mutation model (SMM) (Goldstein et al., 1995). New mutations following this model are obtained by adding or subtracting repeats one by one (Goldstein et al., 1995). These distance matrices were later imported into PAUP 4.0b3a (Swofford, 1998) to build trees with UPGMA (Sokal & Sneath, 1963) and NJ (Saitou & Nei, 1987).

The binary matrix was imported into PAUP 4.0b3a (Swofford, 1998) to produce the distance matrix of Nei and Li (1979) that applies the infinite allele model (IAM), where every mutation produces a new allele of potentially infinite size (Kimura & Crow, 1964). The Nei and Li matrix was used to build UPGMA (Sokal & Sneath, 1963) and NJ (Saitou & Nei, 1987) trees.

TABLE 3

Primer pairs used in the present study. Tag codes refer to fluorescent dyes: B = blue (FAM), G = green (TETRA), Y = yellow (HEX). Primer sequences, target repeat, and linkage group from *Solanum tuberosum* from Milbourne et al. (1998).

Primer	Tag	Primer sequence	Targeted repeat	Size range (bp)	Annealing	Chromosome Temp. (°C)	Percent missing data
stm0003-f	B	gga gaa tca taa caa cca g	(ac)9 (at)9	101–170	48	XII	4%
stm0003-r		aat tgt aac tct gtg tgt gtg					
stm0007-f	B	gga caa gct gtg aag ttt at	(ac)9	185–234	52	XII	58%
stm0007-r		aat tga gaa aga gtg tgt gtg					
stm0014-f	G	cag tct tca gcc cat agg	(gt)5 (at)7 (gt)10	77–201	53	I	18%
stm0014-r		taa aca atg gta gac aag aca aa					
stm0051-f	Y	tac ata cat aca cac acg cg	(ac)7…(ac)7 (at)4	84–116	53	X	23%
stm0051-r		ctg caa ctt ata gcc tcc a					
stm1005-f	B	atg cct ctt acg aat aac tcg g	(gta)6	160–187	59	VIII	27%
stm1005-r		cag cta acg tgg ttg ggg					
stm1017-f	G	gac acg ttc acc ata aa	(att)5	115–137	48	IX	21%
stm1017-r		aga aga ata gca aag caa					
stm1024-f	B	ata cag gac ctt aat ttc ccc aa	(ttg)6	125–152	59	VIII	13%
stm1024-r		tca aaa ccc aat tca atc aaa tc					
stm1029-f	Y	agg ttc act cac aat caa agc a	(c)12	121–166	58	I	41%
stm1029-r		aag att tcc aag aaa ttt gag gg					
stm1041-f	B	gtt gag tag aag gag gat t	(gaa)5	86–101	53	V	13%
stm1041-r		cct ttg tct tct gct ttt g					
stm1049-f	G	cta cca gtt tgt tga ttg tgg tg	(ata)6	176–212	58	I	27%
stm1049-r		agg gac ttt aat ttg ttg gac g					
stm2022-f	B	gcg tca gcg att tca gta cta	(caa)3…(caa)3	167–266	57	II	16%
stm2022-r		ttc agt caa ctc ctg ttg cg					
stm3009-f	B	tca gct gaa cga cca ctg ttc	(tc)13	131–211	63	VII	4%
stm3009-r		gat ttc acc aag cat gga agt c					

RESULTS

MORPHOLOGY

Thirty-five of the 56 characters were significantly different among all taxa (Table 2) as determined by the Tukey-Kramer test and the Likelihood Ratio Chi square test and Pearson Chi square test for qualitative characters in JMP v. 3.1.5 (SAS Institute Inc., 1998) statistical software. The cophenetic correlation coefficient determined that the UPGMA tree constructed with EUCLID had the highest (best) value of r = 0.835, followed by DIST (r = 0.827), MANHAT (r = 0.812), and CORR (r = 0.747). These four similarity matrices with NJ always provided lower r scores. The resulting UPGMA dendrogram (Fig. 2) supported most of the species. We will concentrate discussion of the primitive Mexican diploid species because this morphological study was designed mainly to search for phenetic support of these. Figure 2 is labeled as clusters A–I for ease of discussion.

Cluster A is well defined and includes all species and subspecies in *Solanum* ser. *Bulbocastana* (*Solanum bulbocastanum* subsp. *bulbocastanum*, subsp. *dolichophyllum*, subsp. *partitum*, and *S. clarum*). Both of these species perfectly support Hawkes's (1990) concept of this series. However, *Solanum bulbocastanum* subsp. *dolichophyllum* is intermixed with subspecies *partitum*. Cluster B includes both species in Hawkes's series *Polyadenia* (*S. lesteri*, *S. polyadenium*), likewise providing support for this series.

Clusters C–I include all members of Hawkes's (1990) series *Pinnatisecta*, plus other 2x(1EBN) species from South America in series *Circaeifolia*, *Commersoniana*, *Lignicaulia*, and *S. santolallae* (2x[2EBN], ser. *Conicibaccata*). Cluster C includes *S. cardiophyllum* subsp. *cardiophyllum* and *S.* ×*sambucinum* (both *Solanum* ser. *Pinnatisecta*) and *S. circaeifolium* (ser. *Circaeifolia*). *Solanum* ×*sambucinum* was of putative origin with none of these but with *S. pinnatisectum* and *S. cardiophyllum* subsp. *ehrenbergii* and *S. pinnatisectum*. Cluster D includes *S. pinnatisectum* and *S. lignicaule* (ser. *Lignicaulia*). Cluster E includes species-specific subclusters *S. tarnii* and *S. trifidum* that group together, the putative hybrid *S.* ×*michoacanum* (*S. bulbocastanum* × *S. pinnatisectum*), one "misplaced" or misidentified accession of *S. brachistotrichium*, the sole accession of *S. hintonii* (ser. *Pinnatisecta*), and *S. commersonii* (ser. *Commersoniana*). Cluster F includes both accessions of *S. nayaritense* (ser. *Pinnatisecta*) and the putative hybrid *S. bulbocastanum* × *S. cardiophyllum* (containing members of both ser. *Bulbocastana* and *Pinnatisecta*; Rodríguez et al., 1995). Cluster G contains all accessions of *S. jamesii* (ser. *Pinnatisecta*), and cluster H all accessions of *S. cardiophyllum* subsp. *ehrenbergii* plus one accession of *S. brachistotrichium* (ser. *Pinnatisecta*). Cluster I contains four of the six accessions of *S. brachistotrichium* and both replicate groups of the sole accession of *S. stenophyllidium* (ser. *Pinnatisecta*), but they do not group adjacent to each other.

In summary, the primitive Mexican diploid species are well supported phenetically except for *S. brachistotrichium*. *Solanum stenophyllidium* is problematic, perhaps due to a single accession examined, but groups with most accessions of *S. brachistotrichium*. The putative hybrid *S.* ×*sambucinum* groups between its parents, and the putative hybrids *S.* ×*michoacanum* and *S. bulbocastanum* × *S. cardiophyllum* away from their parents. The principal component analysis (not shown) presents a very similar clustering, or lack of it, regarding *S. brachistotrichium*.

Stepwise discriminant analysis separated all taxa by the following 21 morphological characters (cf. see Fig. 3), arranged from highest to lowest discriminant ability: (1) lateral leaflet decurrency (character 30, Table 2); (2) stem pubescence type (6); (3) spicy odor of leaves (34); (4) presence of interstitial leaflets (12); (5) ratio: length from widest point of most distal lateral leaflet blade to base of blade/length of most distal lateral leaflet blade (21); (6) shape of pseudostipular leaflet (33); (7) abaxial leaf pubescence length (28); (8) ratio: length of second most distal lateral leaflet blade/width of second most distal lateral leaflet (24); (9) presence of stem wings (4); (10) petiolule length of most distal lateral leaflet (22); (11) widest leaflet pair (11); (12) ratio: length of most

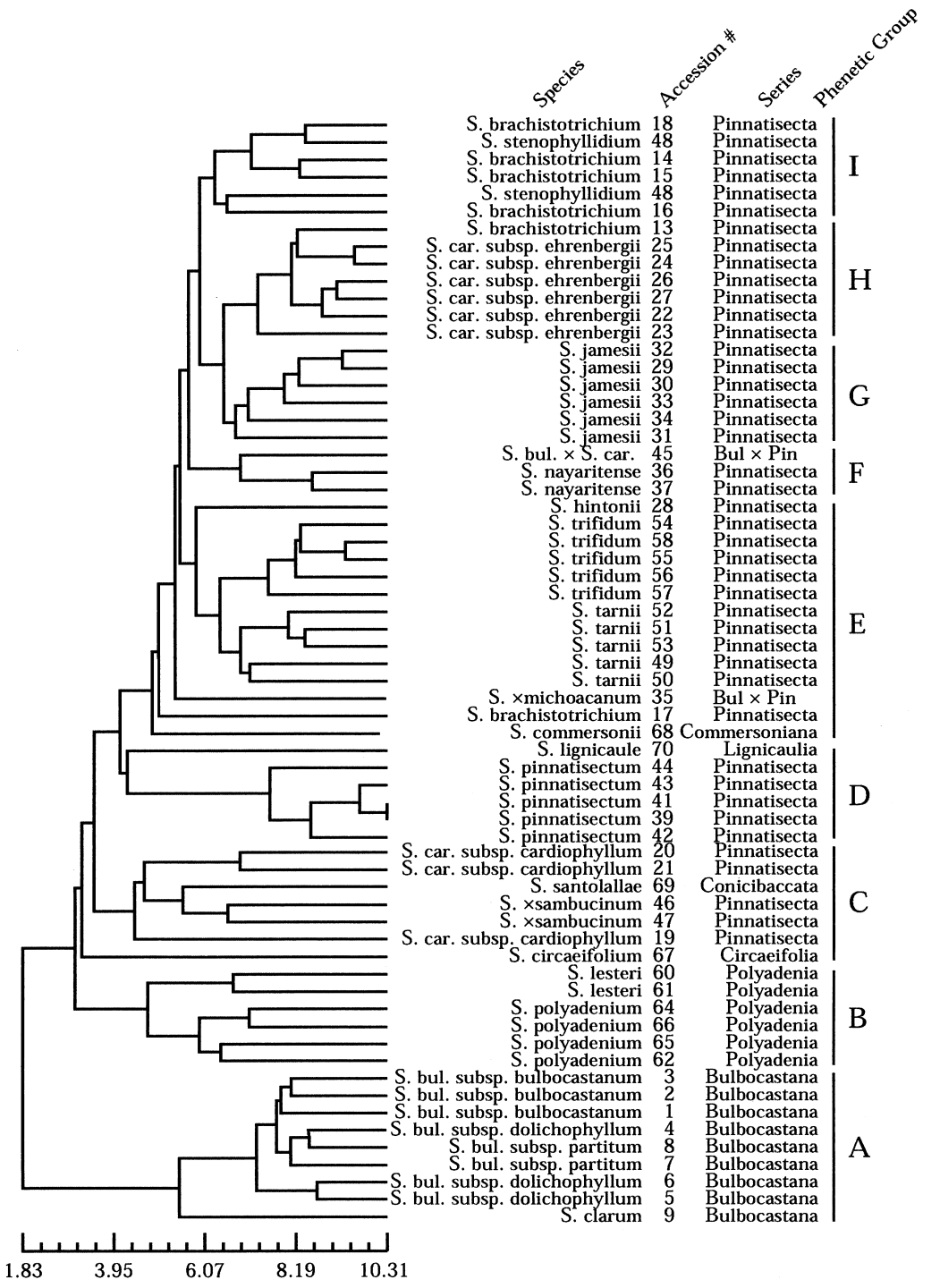

Figure 2. UPGMA dendrogram (Euclidean similarity option) based on 35 of 56 morphological characters that differ significantly among taxa. Species names and accession numbers, as in Table 1 and Figures 1 and 4, and phenetic groups as discussed in the text.

distal lateral leaflet blade/width of most distal lateral leaflet (19); (13) terminal leaflet width (13); (14) petiolule length of second most distal lateral leaflet (27); (15) petiole wings (31); (16) number of leaflet pairs (9); (17) petiolule length of terminal leaflet (17); (18) most distal lateral leaflet width (18); (19) length from widest point of second most distal lateral leaflet blade to base of blade (25); (20) length from widest point of the terminal leaflet blade to base of blade (15); (21) leaf width (7).

We also used stepwise discriminant analysis to discover characters that best distinguished the nine phenetically most similar primitive Mexican diploid species. This analysis used the 14 morphological characters, arranged from highest to lowest discriminant ability. Nine of these 14 are quantitative character distributions and are graphically displayed in Figure 3, while 5 character state distributions are qualitative: (1) fruit shape: globose in all species except *S. hintonii* and *S. trifidum*, which had conical fruits (56); (2) leaf decurrence: all are laterally decurrent except *S. cardiophyllum* subsp. *ehrenbergii, S. hintonii*, and *S. tarnii*, which are not (30); (3) shape of pseudostipular leaf: lunate in all species except pinnulate in *S. jamesii* (33); (4) only *S.* ×*michoacanum* and *S. tarnii* lack interstitial leaflets (12); (5) ratio: length of second most distal lateral leaflet blade/width of second most distal lateral leaflet (24); (6) abaxial leaf pubescence length (28); (7) length of calyx acumen (42); (8) petiole length (32); (9) width of second most distal lateral leaflet (23); (10) secondary color of abaxial surface of corolla: 100% white in *S. hintonii*, *S. stenophyllidium*, *S. tarnii*, and *S. trifidum*, only 42.8% white in *S. brachistotrichium* (the rest white with tones of violet), 16.6% white in *S. cardiophyllum* subsp. *ehrenbergii* and *S. jamesii* (the rest white with tones of violet), and 50% white in *S. nayaritense* (the rest white with tones of violet) (54); (11) style exsertion (46); (12) length from widest point of second most distal lateral leaflet blade to base of blade (25); (13) stem diameter (3); (14) number of leaflet pairs (9).

Significant differences in character states for this reduced matrix of nine phenetically most similar primitive Mexican diploid taxa, as determined by the Tukey-Kramer test, found 21 characters (all quantitative) best distinguish these taxa. These 21 characters include all 9 of the quantitative characters mentioned above as determined by STEPDISC. The means, ranges, and standard deviations of these 21 characters are graphically presented in Figure 3. It shows that most of these characters overlap considerably in range, and provide few species-specific character states useful for easy diagnoses of taxa. Some exceptions, however, are low number of lateral leaflet pairs in *S. nayaritense* (character 9), long petiolules (17), long petioles (32), and wide corolla lobes as measured at the base of the corolla junction (51) in *S. hintonii*. This trend of the sole to predominant presence of overlapping character states to define taxa is common in *Solanum* sect. *Petota* (Spooner & van den Berg, 1992; Spooner & Hijmans, 2001), and termed polythetic support. A polythetic morphological species concept is one where species are defined where they have the greatest number of shared features, no single feature of which is essential for group membership or is sufficient to make an organism a member of a group (Sokal & Sneath, 1963; Stuessy, 1990). Stated otherwise, species are distinguished only by a complex set of character states that overlap in ranges.

MICROSATELLITE PHENETICS

Only 12 of the 45 primers we tried, from 8 linkage groups as mapped by Milbourne et al. (1998) for *Solanum tuberosum*, gave useable results. There were amplification failures in these 12 ranging from 4% in stm0003 and stm3009, to 58% in stm0007, with the average across all primers 22% (Table 3). It is frequent in cross-species amplification to reduce the annealing temperature in order to obtain amplification (Ezenwa et al., 1998). A range of up to 5ºC less was attempted to achieve amplification when no product otherwise could be obtained, but with futile results. We considered an amplification successful when the positive control (*S. tuberosum*) amplified, as well as most of the primitive Mexican diploids.

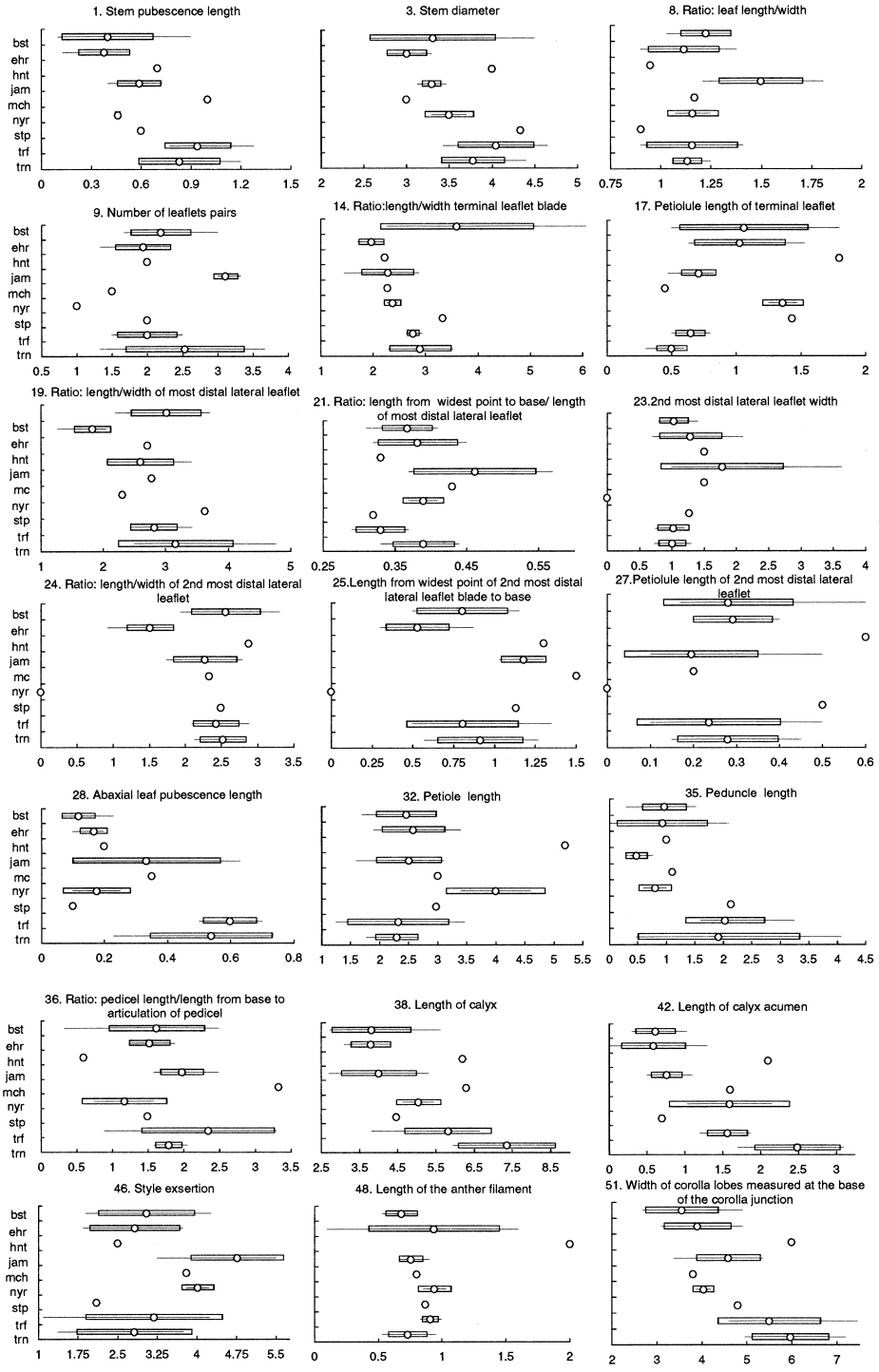

Figure 3. Means (dot), ranges (length of line), and one standard deviation of the mean (length of box) for 21 of the 35 statistically significant morphological characters of the phenetically most similar diploid wild species from the United States, Mexico, and Central America. Included here are only the quantitative characters; the number preceding the character corresponds to Table 2. All measurements are in cm except characters 1, 3, 28, 38, 42, 46, 48, and 51, which are in mm. Three-letter species codes follow Spooner and Hijmans (2001): bst = *S. brachistotrichium*; ehr = *S. cardiophyllum* subsp. *ehrenbergii;* hnt = *S. hintonii;* jam = *S. jamesii;* mch = *S.* ×*michoacanum;* nyr = *S. nayaritense;* stp = *S. stenophyllidium;* trf = *S. trifidum;* trn = *S. tarnii.*

Figure 4. Neighbor-joining dendrogram based on microsatellite data (Nei & Li, 1979, similarity). Species names and accession numbers as in Table 1 and Figures 1 and 2, and phenetic groups from Figure 2.

The trees obtained from the two stepwise mutation model (SMM) matrices showed little to no concordance with the morphology phenogram, that is, they failed to cluster well-defined morphological species (trees not presented). The infinite allele model (IAM) with UPGMA and NJ clustered taxa somewhat better but still very poorly relative to the morphology phenogram (Fig. 2). Our first analysis included all taxa, and NJ clustered taxa best (shown in Fig. 4), but still only provided weak clustering of some accessions of some species (such as a partial clustering of *Solanum cardiophyllum* subsp. *cardiophyllum*, *S. jamesii*, and *S. nayaritense*, all members of ser. *Pinnatisecta*), but with failure to completely cluster these and most other species. To attempt better clustering of putatively related taxa, we analyzed just the most phenetically similar primitive Mexican diploids *S. brachistotrichium*, *S. cardiophyllum* subsp. *cardiophyllum*, subspecies *ehrenbergii*, *S. nayaritense*, and *S. stenophyllidium* (all within ser. *Pinnatisecta*). This reduced analysis (not presented) still intermixed accessions of all species except for *S. cardiophyllum* subsp. *cardiophyllum* as in Figure 4; that is, it still showed little to no species-specific clustering. Microsatellites also were of no use to investigate hybrid origins of *S.* ×*michoacanum*, *S.* ×*sambucinum*, and the putative hybrid *S. bulbocastanum* × *S. cardiophyllum* (Rodríguez & Vargas-P., 1994). There were no additive species-specific bands present to test these hypotheses.

Discussion

CONCORDANCE OF MORPHOLOGICAL DATA WITH PRIOR HYPOTHESES OF RELATIONSHIPS AND CHLOROPLAST DNA DATA

Our morphological results show areas of concordance and discordance with cpDNA results of Spooner and Sytsma (1992), Rodríguez and Spooner (1997), and with prior hypotheses of interspecific relationships (Hawkes, 1989, 1990; Hawkes et al., 1988; Hawkes & Jackson, 1992). For example, all members of *Solanum* ser. *Bulbocastana* are well resolved and separated from the rest of the Mexican diploids (Fig. 2, cluster A). However, *S. bulbocastanum* subsp. *bulbocastanum* was the only subspecies relatively well supported, not *S. bulbocastanum* subsp. *dolichophyllum* and subspecies *partitum*. Our morphological data also unite *S. bulbocastanum* and *S. clarum*, supporting Hawkes (1990) who united them in *Solanum* ser. *Bulbocastana*. Chloroplast DNA data (Spooner & Sytsma, 1992; Rodríguez & Spooner, 1997), however, supported the sister-taxon relationship of *S. bulbocastanum* and *S. cardiophyllum* (Fig. 2, clusters A and C, in part), not *S. bulbocastanum* and *S. clarum*.

Solanum lesteri and *S. polyadenium* (cluster B) form a good phenetic group, concordant with cpDNA results (Spooner & Sytsma, 1992) and Hawkes's (1990) placement in *Solanum* ser. *Polyadenia*. *Solanum* ×*sambucinum* clusters with neither of its putative parents (*S. cardiophyllum* subsp. *ehrenbergii* and *S. pinnatisectum*). *Solanum trifidum* and *S. tarnii* (both ser. *Pinnatisecta*) also form a good phenetic group (cluster E), concordant with cpDNA data, and recognition of their phenetic similarity by Hawkes et al. (1988). Although not examined for cpDNA, Hawkes (1990) intuitively noted a morphological similarity of *S. hintonii* and *S. trifidum*, concordant with our data (cluster E).

The cluster of *Solanum nayaritense* with the putative hybrid of *S. bulbocastanum* × *S. cardiophyllum* (cluster F) is discordant with morphological interpretations (Rodríguez et al., 1995) as *S. nayaritense* has not been suggested as its progenitor. The phenetic relationship of *S. jamesii* (cluster G) and *S. cardiophyllum* subsp. *ehrenbergii* (cluster H) has never been suggested before, but is reasonable based on cpDNA results that show them to be part of the same clade (Spooner & Sytsma, 1992; Rodríguez & Spooner, 1997). The phenetic separation of *S. cardiophyllum* subsp. *ehrenbergii* (cluster H) from subspecies *cardiophyllum* (cluster C) is concordant with cpDNA data (Rodríguez & Spooner, 1997). The phenetic similarity of *S. brachistotrichium* and *S. stenophyllidium* (cluster I) also has support with cpDNA data (Spooner & Sytsma, 1992), which places them on the same clade.

MICROSATELLITE DATA ANALYSIS

There are two divergent hypotheses of microsatellite evolution and its reflection on best analytical methods for assessment of genetic distances and phylogeny. Schlötterer and Tautz (1992) have shown slippage during replication, which would support the use of the simple mutation model (SMM) for analyses. However, Colson and Goldstein (1999) studied microsatellite loci in *Drosophila* and found that many mutation events in microsatellites are more complex than assumed by SMM. They advised the use of sequence data to assess the accumulation of imperfections within the microsatellite repeat, reducing the number of consecutive repeats. These imperfections can be insertions, deletions, or base substitutions, which, along with stepwise mutation drift to smaller sizes, have contributed to degradation of microsatellite loci over time. The authors caution against the use of microsatellite size alone for population or phylogenetic relationships (Colson & Goldstein, 1999). The SMM has been supported by phylogeny reconstructions in bovine species (MacHugh et al., 1997), fish (Ruzzante, 1998), and human populations (Valdes et al., 1993; Shriver et al., 1995; Kimmel et al., 1996). On the other hand, the infinite allele model (IAM) has been supported for data sets with numerous compound microsatellites (Estoup et al., 1995), which would be the case in our data set. In this study, the best concordance with morphological data was produced with the IAM and the worst with the SMM, but both were very poor. The IAM was the most useful in analyzing subspecies differences in *S. tuberosum* (Raker & Spooner, 2002).

MICROSATELLITE CROSS-SPECIES AMPLIFICATION

Microsatellites are expensive to develop. One of their appealing features is their potential use in related groups beyond the mainly economic plants where they were developed. There are several examples of successful amplification across species. Microsatellites distinguished two closely related species in genus *Sitobion* (Hemiptera: Aphidoidea) (Figueroa et al., 1999). Microsatellites developed for the tropical tree *Pithecellobium elegans* were conserved in several species of the tribe Ingeae (Dayanandan et al., 1997). Microsatellites from peach successfully amplified fragments in apple, suggesting that they might be useful as well in other fruit crops (Cipriani et al., 1999). In the genus *Dianthus*, microsatellite loci developed from the EMBL database were tested in 26 species within the genus, and successful amplification implied that the species are closely related or that the primer regions are well conserved (Smulders et al., 2000). Eight of 17 microsatellite primer pairs designed for *Quercus petraea* were successful in producing amplifications in the related genus *Castanea*, and 4 of the 17 in *Fagus*. Twelve of these 136 amplifications were cloned and sequenced and shown to be homologous to *Quercus petraea*; nevertheless, the authors recognized a tendency for decreasing ability to successfully amplify loci, with increasing evolutionary distance across the family (Steinkellner et al., 1997). The same relationship between an increasing genetic distance and decreasing success of microsatellite amplification was observed in cassava (*Manihot esculenta* Crantz), through tests with six *Manihot* wild species (Roa et al., 2000).

In addition to problems producing microsatellite fragments across species, there also are problems with homology of the microsatellite fragment that is necessary for correct phylogenetic interpretations (Wendel & Doyle, 1998). For successful amplification of homologous fragments the target sequence for the primers *and* flanking sequence about the microsatellites must be sufficiently conserved (Ezenwa et al., 1998). Knowledge of these criteria is costly as it requires sequencing the PCR fragments.

In our study the primers were developed through a Genbank search of *Solanum tuberosum* (Milbourne et al., 1998). Chloroplast DNA data (Spooner & Sytsma, 1992) show the primitive Mexican diploids to be the most distantly related group to *S. tuberosum* in section *Petota*, and microsatellites performed poorly for phylogenetic reconstructions. Similar problems have been found in other groups. Sequence variation and/or variation in the number of repeats has been

found by Peakall et al. (1998), who used 31 soybean microsatellite loci in wild relatives and found that cross-species amplification was low, mainly in closely related genera. When cross-species amplification was achieved there was sequence variation in the flanking regions and within the repeats (non-homologous amplification). Westman and Kresovich (1998) tried to use *Arabidopsis* primer pairs to amplify microsatellites in the closely related genus *Brassica*, but many amplified loci did not hybridize with microsatellite probes, and probably did not contain repeats. In animals, microsatellites derived from two species of wasps successfully cross-species amplified in different related genera from the Vespidae. However, the loci from one species were more conserved than those from the other, warning that it is difficult to reach generalizations regarding conservation rates using data from single species (Ezenwa et al., 1998). In avian microsatellites, 73 primer sets from 16 species across nine families were tested on *Quelea* species. Only 22 of these 73 pairs produced homologous amplification (Dallimer, 1999).

The issue of cross-species amplification is complex. As seen above, it can be achieved across genera within families, or not even occur across species within a genus. To our knowledge there is no comprehensive summary that addresses the comparative extent of microsatellite conservation or whether the failure of cross-species amplification is due to primer design or phylogenetic distance. Clisson et al. (2000) sequenced primate microsatellites with primers designed in humans and found that alterations in the base composition of repeats, and insertions and deletions in flanking regions, are important components of the variation but introduced homoplasy (same size but different composition) reducing their phylogenetic utility.

CONCLUSIONS

Most of the species examined in our study were supported by morphological characters, except *Solanum brachistotrichium* and possibly *S. stenophyllidium*. However, microsatellites provided poor to no support for species. This could be caused by non-homology of repeat length between the primers, or in our study by not having enough data to provide phylogenetic signal. There continue to be unanswered questions about microsatellites regarding mutation processes, proper analytical tools for phylogeny, and knowledge of the extent of cross-species amplification. Our results show that microsatellites developed from *S. tuberosum* have reduced utility to establish good morphological species from the phylogenetically distant United States, Mexican, and Central American diploid wild potato species. Lara-Cabrera and Spooner (2004) addressed the validity of these species and their interrelationships with AFLP data.

NOTE IN PROOF

These results, and those of Lara-Cabrera and Spooner (2004), were instrumental in revising the wild potatoes of North and Central America (Spooner et al., 2004).

ACKNOWLEDGMENTS

We thank John Bamberg and staff of the United States Potato Genebank for germplasm and locality data; Charles Nicolet and staff of the University of Wisconsin Biotechnology Center for technical help; Lynn Hummel and staff at Walnut Street Greenhouse for help in growing plants; lab partners Brian Karas, Iris Peralta, Celeste Raker, and Sarah Stephenson for technical advice; Peter Crump for statistical advice; and Robert Giannattasio for artwork. We gratefully acknowledge the long collaboration of Bill D'Arcy, who showed great interest and support to us in this problem and many other issues in the Solanaceae. This study was supported by CONACYT (Mexico) scholarship number 116742 granted to Sabina I. Lara-Cabrera, and by the United States Department of Agriculture.

LITERATURE CITED

Arens, P., P. Odinot, A. W. Van Heusden, P. Lindhout & B. Vosman. 1995. GATA- and GACA-repeats are not evenly distributed throughout the tomato genome. Genome 38: 84–90.

Areshchenkova, T. & M. W. Ganal. 1999. Long tomato microsatellites are predominantly associated with centromeric regions. Genome 42: 536–544.

Bichara, M., I. Pinet, S. Schumacher & R. P. P. Fuchs. 2000. Mechanisms of dinucleotide repeat instability in *Escherichia coli.* Genetics 154: 533–542.

Cipriani, G., G. Lot, W. G. Huang, M. T. Marrazzo, E. Peterlunger & R. Testolin. 1999. AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L.) Batsch]: Isolation, characterisation and cross-species amplification in *Prunus.* Theor. Appl. Genet. 99: 65–72.

Clisson, I., M. Lathuilliere & R. B. Crouau. 2000. Conservation and evolution of microsatellite loci in primate taxa. Amer. J. Primatol. 50: 205–214.

Colson, I. & D. G. Goldstein. 1999. Evidence for complex mutations at microsatellite loci in *Drosophila.* Genetics 152: 617–627.

Dallimer, M. 1999. Cross-species amplification success of avian microsatellites in the red-billed quelea *Quelea quelea.* Molec. Ecol. 8: 395–398.

Dayanandan, S., K. S. Bawa & R. Kesseli. 1997. Conservation of microsatellites among tropical trees (Leguminosae). Amer. J. Bot. 84: 1658–1663.

Estoup, A., L. Garnery, M. Solignac & J. M. Cournet. 1995. Microsatellite variation in honeybee (*Apis mellifera* L.) populations: Hierarchical genetic structure and test of the infinite allele and stepwise mutation models. Genetics 140: 679–695.

Ezenwa, V. O., J. M. Peters, Y. Zhu, E. Arevalo, M. Hastings, P. Seppa, J. S. Pedersen, F. Zacchi, D. Queller & J. E. Strassmann. 1998. Ancient conservation of trinucleotide microsatellite loci in polistine wasps. Molec. Phylogenet. Evol. 10: 168–177.

Figueroa, C., J. C. Simon, J. F. Le Gallic & H. M. Niemeyer. 1999. Molecular markers to differentiate two morphologically close species of the genus *Sitobion.* Entomol. Exp. Appl. 92: 217–225.

Goldstein, D. B., A. Ruiz-Linares, L. L. Cavalli-Sforza & M. W. Feldman. 1995. An evaluation of genetic distances for use with microsatellite loci. Genetics 139: 463–471.

Hanneman, R. E., Jr. 1994. Assignment of Endosperm Balance Numbers to the tuber-bearing Solanums and their close non-tuber bearing relatives. Euphytica 74: 19–25.

Hawkes, J. G. 1989. Nomenclatural and taxonomic notes on the infrageneric taxa of the tuber-bearing Solanums (Solanaceae). Taxon 38: 489–492.

—. 1990. The Potato: Evolution, Biodiversity and Genetic Resources. Belhaven Press, London.

— & M. T. Jackson. 1992. Taxonomic and evolutionary implications of the Endosperm Balance Number hypothesis in potatoes. Theor. Appl. Genet. 84: 180–185.

—, J. P. Hjerting & T. R. Tarn. 1988. Two new tuber-bearing *Solanum* species from Mexico: *S. tarnii* and *S. matehuelae.* Phytologia 65: 114–118.

Kardolus J. P. 1998. A Biosystematic Analysis of *Solanum acaule.* Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Kimmel, M., R. Chakraborty, D. N. Stivers & R. Deka. 1996. Dynamics of repeat polymorphisms under a forward-backward mutation model: Within and between-population variability at microsatellite loci. Genetics 143: 549–555.

Kimura, M. & J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. Genetics 143: 549–555.

Kostia, S., M. Ruohonen-Lehto, R. Väinölä & S. L. Varvio. 2000. Phylogenetic information in inter-SINE and inter-SSR fingerprints of the *Artiodactyla* and evolution of the BovtA SINE. Heredity 84: 37–45.

Lara-Cabrera, S. & D. M. Spooner. 2004. Taxonomy of Mexican diploid wild potato (*Solanum* sect. *Petota*) species: AFLP data. Pl. Syst. Evol. 248: 129–142.

Lin, H. & M. A. Walker. 1998. Identifying grape rootstocks with simple sequence repeat (SSR) DNA markers. Amer. J. Enol. Vitic. 49: 403–407.

Lorieux, M., M. N. Ndjiondjop & A. Ghesquiere. 2000. A first interspecific *Oryza sativa* × *Oryza glaberrima* microsatellite based genetic linkage map. Theor. Appl. Genet. 100: 593–601.

MacHugh, D. E., M. D. Shriver, R. T. Loftus, P. Cunningham & D. G. Bradley. 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of Taurine and Zebu cattle (*Bos taurus* and *Bos indicus*). Genetics 146: 1071–1086.

Milbourne, D., R. Meyer, J. E. Bradshaw, E. Baird, N. Bonar, J. Provan, W. Powell & R. Waugh. 1997. Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. Molec. Breed. 3: 127–136.

—, —, A. J. Collins, L. D. Ramsay, C. Gebhardt & R. Waugh. 1998. Isolation, characterization and mapping of simple sequence repeat loci in potato. Molec. Gen. Genet. 259: 233–245.

Minch, E., A. Ruiz-Linares, D. Goldstein, M. Feldman & L. L. Cavalli-Sforza. 1997. Microsat v. 1.5d: A computer program for calculating various statistics on microsatellite allele data. <http://lotka.stanford.edu/microsat.html>.

Nader, W., D. Werner & M. Wink. 1999. Genetic diversity of scarlet macaws *Ara macao* in reintroduction studies for threatened populations in Costa Rica. Biol. Conservation 87: 269–272.

Nei, M. & W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. U.S.A. 76: 5269–5273.

Peakall, R., S. Gilmore, W. Keys, M. Morgante & A. Rafalski. 1998. Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: Implications for the transferability of SSRs in plants. Molec. Biol. Evol. 15: 1275–1287.

Petren, K., B. R. Grant & P. R. Grant. 1999. A phylogeny of Darwin's finches based on microsatellite DNA length variation. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 266: 321–329.

Powell, W., G. C. Machray & J. Provan. 1996. Polymorphism revealed by simple sequence repeats. Trends Pl. Sci. 1: 215–222.

Provan, J., W. Powell & R. Waugh. 1996a. Analysis of cultivated potato *(Solanum tuberosum)* using intermicrosatellite amplification. Genome 39: 767–769.

—, — & —. 1996b. Microsatellite analysis of relationships within cultivated potato *(Solanum tuberosum).* Theor. Appl. Genet. 92: 1078–1084.

Raker, C. M. & D. M. Spooner. 2002. An examination of the *Solanum tuberosum* subspecies *tuberosum* and subspecies *andigena* using microsatellite DNA. Crop Sci. 42: 1451–1458.

Rieseberg, L. H. & S. J. Brunsfeld. 1992. Molecular evidence and plant introgression. Pp. 151–176 *in* P. S. Soltis, D. E. Soltis & J. J. Doyle (editors), Molecular Systematics of Plants. Chapman & Hall, New York.

Roa, A. C., P. Chavarriaga-Aguirre, M. C. Duque, M. M. Maya, M. W. Bonierbale, C. Iglesias & J. Tohme. 2000. Cross-species amplification of cassava *(Manihot esculenta)* (Euphorbiaceae) microsatellites: Allelic polymorphism and degree of relationship. Amer. J. Bot. 87: 1647–1655.

Rodríguez, A. & D. M. Spooner. 1997. Chloroplast DNA analysis of *Solanum bulbocastanum* and *S. cardiophyllum* subsp. *ehrenbergii* (sect. *Petota*). Syst. Bot. 22: 31–34.

— & O. Vargas-P. 1994. Las especies de papa silvestre (*Solanum* L. sección *Petota* Dumortier) en Jalisco. Bull. Inst. Bot, IBUG 2: 1–2.

—, —, E. Villegas & D. M. Spooner. 1995. Wild potato (*Solanum* sect. *Petota*) germplasm collecting expedition to Mexico in 1993, with special reference to *Solanum bulbocastanum* Dunal and *S. cardiophyllum* Lindley. Potato Res. 38: 47–52.

Rohlf, F. J. 1992. NTSYS-pc v.1.70, Numerical Taxonomy and Multivariate Analysis System. Applied Biostatistics, New York.

Rubinsztein, D., B. Amos & G. Cooper. 1999. Microsatellite and trinucleotide-repeat evolution: Evidence for mutational bias and different rates of evolution in different lineages. Philos. Trans., Ser. B. 354: 1095–1099.

Russell, J., J. Fuller, G. Young, B. Thomas, G. Taramino, M. Macaulay, R. Waugh & W. Powell. 1997. Discriminating between barley genotypes using microsatellite markers. Genome 40: 442–450.

Ruzzante, D. E. 1998. A comparison of several measures of genetic distance and population structure with microsatellite data: Bias and sampling variance. Canad. J. Fish. Aquatic Sci. 55: 1–14.

Saitou, N. & M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molec. Biol. Evol. 4: 406–425.

SAS Institute Inc. 1998. SAS Proprietary Software Version 7. SAS Institute, Carry, North Carolina.

Schlötterer, C. & D. Tautz. 1992. Slippage synthesis of simple sequence DNA. Nucl. Acids Res. 20: 211–215.

Schneider, K. & D. S. Douches. 1997. Assessment of PCR-based simple sequence repeats to fingerprint North American potato cultivars. Amer. Potato J. 74: 149–160.

Shriver, M. D., L. Jin, E. Boerwinkle, R. Deka, R. E. Ferrell & R. Chakraborty. 1995. A novel genetic distance for highly polymorphic tandem repeat loci. Molec. Biol. Evol. 12: 914– 920.

Smulders, M. J. M., G. Bredemeijer, W. Rus-Kortekaas, P. Arens & B. Vosman. 1997. Use of short microsatellites from database sequences to generate polymorphism among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor. Appl. Genet. 94: 264–272.

—, W. Rus-Kortekaas & B. Vosman. 2000. Microsatellite markers useful throughout the genus *Dianthus.* Genome 43: 208–210.

Sokal, R. R. & P. H. Sneath. 1963. Principles of Numerical Taxonomy. Freeman, San Francisco, California.

Spooner, D. M. & T. R. Castillo. 1997. Reexamination of series relationships of South American wild potatoes (Solanaceae: *Solanum* sect. *Petota*): Evidence from chloroplast DNA restriction site variation. Amer. J. Bot. 84: 671–685.

— & R. J. Hijmans. 2001. Potato systematics and germplasm collecting, 1989 –2000. Amer. J. Potato Res. 78: 237–268; 395.

— & K. J. Sytsma. 1992. Reexamination of the series relationships of Mexican and Central American wild potatoes (*Solanum* sect. *Petota*): Evidence from chloroplast DNA restriction site variation. Syst. Bot. 17: 432–448.

—, — & E. Conti. 1991. Chloroplast DNA evidence for genome differentiation in wild potatoes (*Solanum* sect. *Petota*: Solanaceae). Amer. J. Bot. 78: 1354–1366.

— & R. G. Van Den Berg. 1992. Species limits and hypotheses of hybridization of *Solanum berthaultii* Hawkes and *S. tarijense*

Hawkes: Morphological data. Taxon 41: 685–700.

— , R. G. Van Den Berg, A. Rodríguez, J. Bamberg, R. J. Hijmans & S. l. Lara-Cabrera. 2004. Wild Potatoes (*Solanum* section *Petota*) of North and Central America. Syst. Bot.Monogr. 68: 1–209 + 9 pl.

Steinkellner, H., C. Lexer, E. Turetschek & J. Glössl. 1997. Conservation of (GA)n microsatellite loci between *Quercus* species. Molec. Ecol. 6: 1189–1194.

Stuessy, T. F. 1990. Plant Taxonomy: The Systematic Evaluation of Comparative Data. Columbia Univ. Press, New York.

Swofford, D. L. 1998. PAUP*, Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b3a. Sinauer, Sunderland, Massachusetts.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucl. Acids Res. 17: 6463–6471.

Teulat, B., C. Aldam, R. Trehin, P. Lebrun, J. H. A. Barker, G. M. Arnold, A. Karp, L. Baudouin & F. Rognon. 2000. An analysis of genetic diversity in coconut (*Cocos nucifera*) populations from across the geographic range using sequence-tagged microsatellites (SSRs) and AFLPs. Theor. Appl. Genet. 100: 764–771.

Valdes, M., M. Slatkin & N. B. Freimer. 1993. Allele frequencies at microsatellite loci: The stepwise mutation model revisited. Genetics 133: 737–749.

Veilleux, R. E., L. Y. Shen & M. M. Paz. 1995. Analysis of the genetic composition of anther-derived potato by randomly amplified polymorphic DNA and simple sequence repeats. Genome 38: 1153–1162.

Wendel, J. F & J. J. Doyle. 1998. Phylogenetic incongruence: Window into genome history and molecular evolution. Pp. 265–296 *in* D. E. Soltis, P. S. Soltis & J. J. Doyle (editors), Molecular Systematics of Plants II: DNA Sequencing. Kluwer Academic, Norwell, Massachusetts.

Westman, A. L. & S. Kresovich. 1998. The potential for cross-taxa simple-sequence repeat (SSR) amplification between *Arabidopsis thaliana* L. and crop brassicas. Theor. Appl. Genet. 96: 272–281.

Winter, P., T. Pfaff, S. M. Udupa, B. Huettel, P. C. Sharma, S. Sahi, R. Arreguin-Espinoza, F. Weigand, F. J. Muehlbauer & G. Kahl. 1999. Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. Molec. Gen. Genet. 262: 90–101.