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(Sect. *Petota*)**

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Chloroplast DNA Analysis of *Solanum bulbocastanum* and *S. cardiophyllum*, and Evidence for the Distinctiveness of *S. cardiophyllum* subsp. *ehrenbergii* (sect. *Petota*)

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ABSTRACT. Chloroplast DNA restriction site analysis was used to test hypotheses of sister group relationships of the diploid Mexican wild potato species *Solanum bulbocastanum* (ser. *Bulbocastana*) and *S. cardiophyllum* (ser. *Pinnatisecta*). A prior chloroplast DNA study including two accessions each of these two species supported them as sister taxa, widely separated from their presumed closest Mexican diploid species relatives. The present study samples more widely by examining 28 accessions of all three subspecies of *S. bulbocastanum*, and 20 accessions of all three subspecies of *S. cardiophyllum*. The results support two main clades exclusive of the outgroup: 1. *Solanum bulbocastanum* (all subspecies) and *S. cardiophyllum* (all subspecies except subsp. *ehrenbergii*), and 2. *S. cardiophyllum* subsp. *ehrenbergii* and members of ser. *Pinnatisecta* other than subsp. *ehrenbergii*. In the first clade, there was little resolution and some chloroplast types were shared by three subspecies of *S. bulbocastanum* and two subspecies of *S. cardiophyllum*. These results, in combination with an earlier chloroplast DNA study of the Mexican and Central American species, suggest that subsp. *ehrenbergii* is related to other members of ser. *Pinnatisecta*, possibly *S. brachistotrichum* or *S. stenophyllidium*, or alternatively that subsp. *ehrenbergii* obtained the chloroplast genome of a species in ser. *Pinnatisecta* by introgression.

Solanum L. sect. *Petota* Dumortier, the potato and its wild relatives, contains 232 species, according to the latest taxonomic interpretation (Hawkes 1990). The group is restricted to the Americas, occurring from southwestern United States to south central Chile. Within sect. *Petota*, there are widely different philosophies and taxonomic practices regarding species circumscriptions, the rank of infraspecific taxa, the assignment of species into series, and hypotheses regarding the extent and evolutionary significance of natural interspecific hybridization (Spooner and Sytsma 1992; Spooner and van den Berg 1992). The series concepts used here are those of the latest comprehensive treatment of sect. *Petota* by Hawkes (1990).

A chloroplast DNA (cpDNA) study of 41 accessions of 28 Mexican and Central American species of sect. *Petota*, including two accessions each of *S. bulbocastanum* Dunal [ser. *Bulbocastana* (Rydb.) Hawkes] and *S. cardiophyllum* Lindley [ser. *Pinnatisecta* (Rydb.) Hawkes], and seven other South American species (Spooner and Sytsma 1992; Fig. 1), showed an unexpected result. *Solanum bulbocastanum* and *S. cardiophyllum* were supported as sister taxa, widely separated from all other Mexican diploid species with which they were thought to be closely related in ser. *Bulbocastana* (also containing

S. clarum Correll) and ser. *Pinnatisecta* [also containing *S. brachistotrichum* (Bitter) Rydb., *S. hintonii* Correll, *S. jamesii* Correll, *S. × michoacanum* (Bitter) Rydb., *S. nayaritense* (Bitter) Rydb., *S. pinnatisectum* Dunal, *S. stenophyllidium* Bitter, *S. tarnii* Hawkes and Hjert., and *S. trifidum* Correll].

Solanum bulbocastanum grows from west central Mexico to western Guatemala (Fig. 2), from 1,400 to 2,350 m, in generally open situations of oak forests and tropical deciduous forests. *Solanum cardiophyllum* grows from west central Mexico to south central Mexico (Fig. 3), from 1,100 to 2,600 m, in open situations of tropical deciduous forests and mesquite-cactus grasslands, and as a weed in corn and bean fields (Correll 1962; Luna et al. 1988; Hawkes 1990; Rodríguez 1991). The two species have widely overlapping ranges in central Mexico.

Neither morphological, crossing, nor immunological data provided a clue to the sister group relationships of *S. bulbocastanum* and *S. cardiophyllum*, and they always have been placed in different series (Rydberg 1924; Correll 1962; Hawkes 1990). They are very different vegetatively from each other, with *S. bulbocastanum* having entire leaves and *S. cardiophyllum* having pinnately dissected leaves. Although *S. bulbocastanum* and *S. cardiophyllum* can be hybridized with each other artificially to

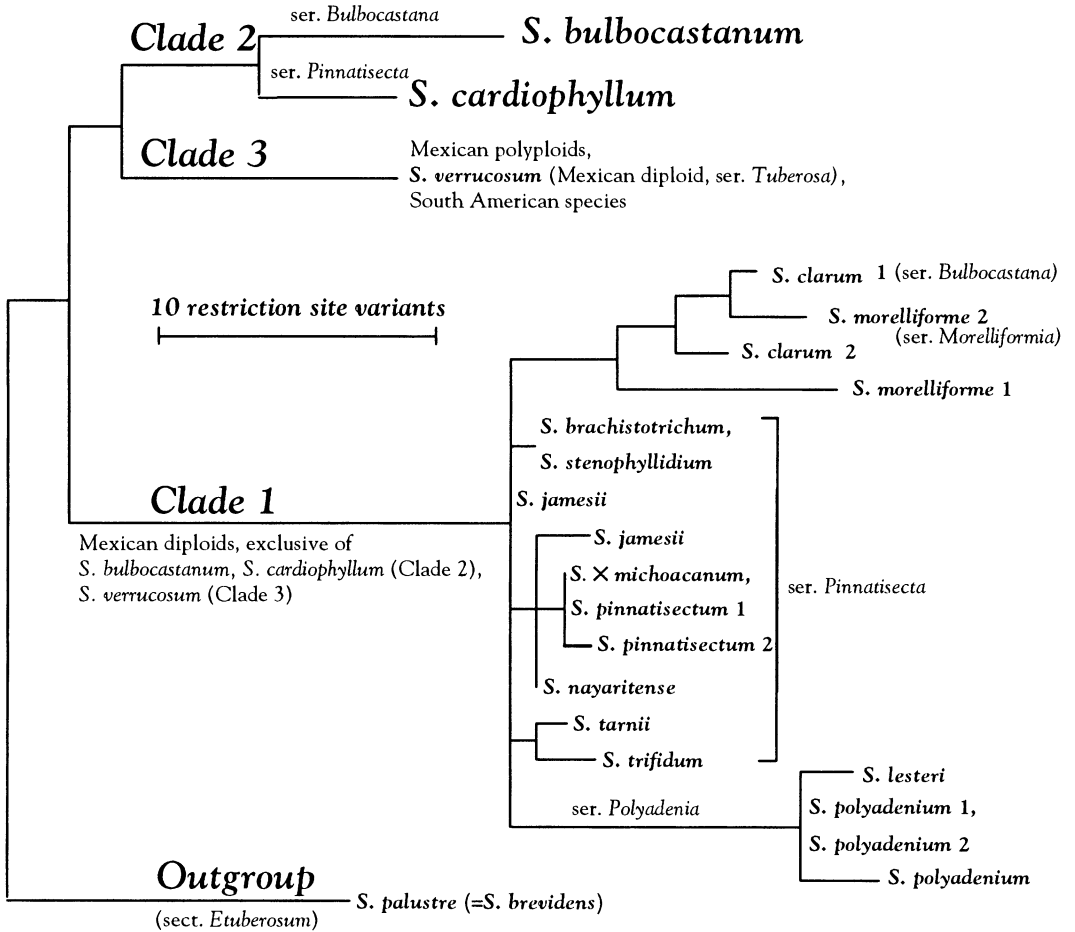
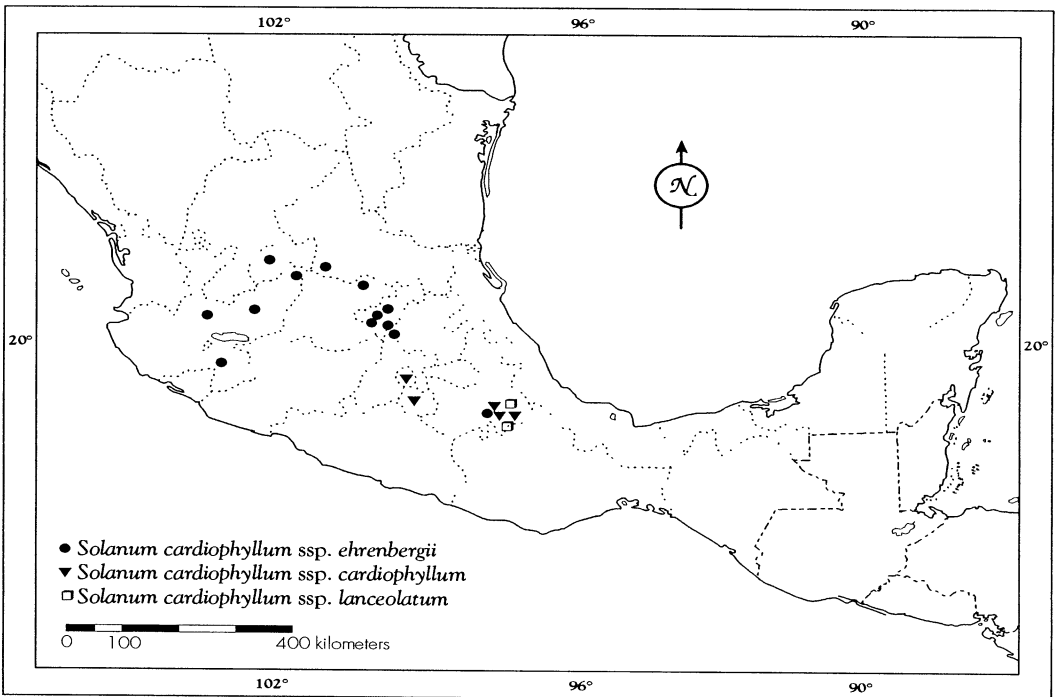
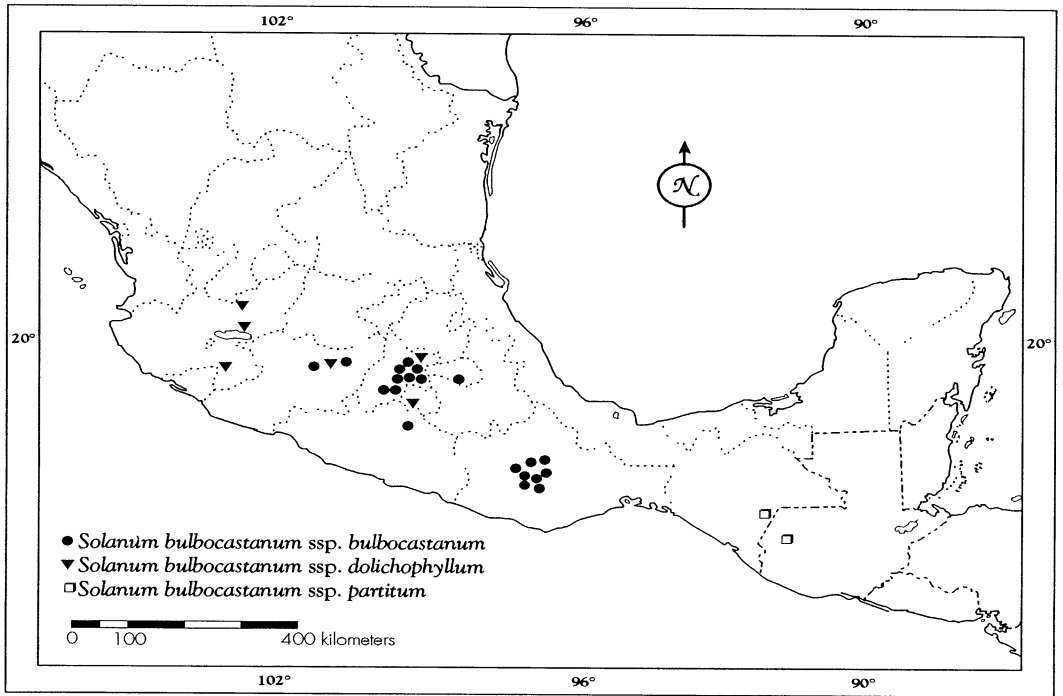


FIG. 1. Abstracted cladistic results (as a phylogram) of 41 accessions of 28 Mexican and Central American species and seven other South American species of sect. *Petota* (Spooner and Sytsma 1992), showing the three major clades. Clade 1 is unmodified; Clade 2 shows only one of each accession of *Solanum bulbocastanum* and *S. cardiophyllum*, and Clade 3 is greatly simplified, showing only the length of the first branch of the clade. Series affiliations follow Hawkes (1990).

form F_1 individuals with high pollen stainability, and fertile F_2 plants can be obtained from these, equally fertile artificial hybrids have been obtained with both species and other species in ser. *Pinnatisecta* (Magoon et al. 1958; Graham et al. 1959; Graham and Dionne 1961). In contrast, the other species in Clade 1 (Fig. 1) of ser. *Bulbocastana* (*S. clarum*), ser. *Morelliformia* Hawkes (*S. morelliforme* Bitter and G. Muench), and ser. *Polyadenia* Correll (*S. lesteri* Hawkes and Hjert., *S. polyadenium* Rydb.) are cross-incompatible with *S. bulbocastanum*, *S. cardiophyllum*, and other members of ser. *Pinnatisecta* (Hawkes 1990; Hanneman 1994). Crossing data, therefore, would not have suggested *S. bulbocastanum* and *S. cardiophyllum* to be in a distinct clade from other members of ser. *Pinnati-*

secta. Immunological studies (Gell et al. 1960) also suggested that *S. bulbocastanum*, *S. cardiophyllum*, and other members of ser. *Pinnatisecta* were equally related.

Besides questions of affiliations of *S. bulbocastanum* and *S. cardiophyllum* to series, there is another question regarding their division into varieties or subspecies. Both species have been divided by various authors into three or four varieties or subspecies each. Rydberg (1924) and Correll (1962) recognized *S. ehrenbergii* (Bitter) Rydb. as a distinct species, while Hawkes (1990) classified *S. ehrenbergii* as a subspecies of *S. cardiophyllum* (subsp. *ehrenbergii* Bitter). A comparison of differing classifications of Correll (1962) and Hawkes (1990) is presented in Fig. 4. Preliminary field observations



FIGS. 2-3. Distribution of the examined populations of *Solanum bulbocastanum* and *S. cardiophyllum*; these cover most of the known range of the species (Correll 1962; Hawkes 1966, 1990).

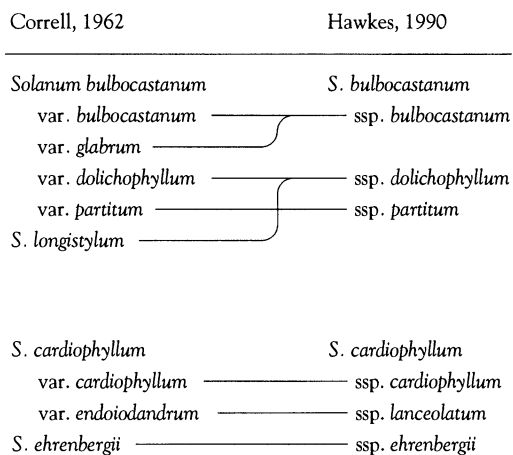


FIG. 4. A comparison of the classifications of infraspecific taxa of *Solanum bulbocastanum* and *S. cardiophyllum* of Correll (1962) and Hawkes (1990).

(Spooner et al. 1991; Rodríguez et al. 1995) showed us overlapping ranges of character states that led us to question whether the subspecies were worthy of taxonomic recognition.

This study expands the prior cpDNA study (Spooner and Sytsma 1992), by characterizing more accessions, including all subspecies, of *S. bulbocastanum* and *S. cardiophyllum*. The objectives were to use cpDNA restriction site analysis to determine if the unexpected sister group relationship of *S. bulbocastanum* and *S. cardiophyllum* was maintained with these many additional accessions, and to see if cpDNA was of value to distinguish the subspecies.

MATERIALS AND METHODS

Plants. Twenty-eight accessions of *S. bulbocastanum* including all subspecies, twenty accessions of *S. cardiophyllum* including all subspecies, and one accession each of *S. pinnatisectum* and *S. palustre* D.F.L. Schlechtendal were analyzed for cpDNA variation (Table 1). Based on results of Spooner et al. (1993), *S. palustre* was used as the outgroup [*Solanum brevidens* Phil. included in that paper is now recognized as a synonym of *S. palustre* (Contreras and Spooner in press)]. To illustrate the concordance of this study with Spooner and Sytsma (1992; Fig. 1), the cpDNA data from *S. brachistotrichum* and *S. stenophyllum* were used in the cladistic analysis. Accessions were obtained from the United States potato germplasm bank of the National Research Support Program-6 (NRSP-6) at Sturgeon Bay, Wisconsin (Bamberg et al. 1996) and

from a 1993 expedition to Mexico that concentrated on *S. bulbocastanum* and *S. cardiophyllum* (Rodríguez et al. 1995).

Identities of the NRSP-6 accessions were provided by visiting taxonomists to NRSP-6 (Hanneman 1989); we personally identified our 1993 collections of *S. bulbocastanum* and *S. cardiophyllum* (Rodríguez et al. 1995). The rare taxa *S. bulbocastanum* subsp. *partitum* (Correll) Hawkes and *S. cardiophyllum* subsp. *lanceolatum* (P. Berthault) Bitter could only be represented by two accessions each (Table 1). These accessions represent the greatest geographic and subspecies diversity available from NRSP-6 and recent field work. Voucher specimens are deposited at the Mexican National Potato Program in Toluca, Mexico, the University of Guadalajara Herbarium (IBUG), and the United States Potato Introduction Herbarium (PTIS; Bamberg and Spooner 1994).

Chloroplast DNA. Pooled fresh leaves of ten plants per accession of the NRSP-6 germplasm accessions were collected from 2-month-old plants for DNA extraction. DNA of the 1993 collections in Mexico was extracted from ten pooled leaves collected in the field and preserved in silica gel (Chase and Hills 1991). We pooled leaves to ensure sufficient DNA for this study. This was done especially for *S. bulbocastanum* that usually gives a very dark DNA extract that does not always cut well with restriction endonucleases without CsCl purification. Preparations of total DNA were made from about 5 g of fresh or 2 g of dried leaf tissue following the procedure of Doyle and Doyle (1987), substituting 6× CTAB for 2× CTAB (Smith et al. 1991). DNA was purified over CsCl/ethidium bromide gradients. Restriction endonuclease digestions, agarose-gel electrophoresis, unidirectional Southern transfers to nylon membranes (Bio-trans™), and filter hybridization followed methods in Palmer (1986). DNA samples were digested with 20 restriction endonucleases: *Ava*I, *Bam*HI, *Ban*II, *Bcl*II, *Bgl*III, *Bst*NI, *Cla*I, *Dra*I, *Eco*O109, *Eco*RI, *Eco*RV, *Hinc*II, *Hind*III, *Hpa*II, *Hph*I, *Nci*I, *Nsi*I, *Sst*I, *Xba*I, and *Xho*I. We used the restriction endonucleases used in the prior global analysis of Mexican and Central American species (Spooner and Sytsma 1992) to have comparable data for this nested study of *S. bulbocastanum* and *S. cardiophyllum*. Exceptions were the omission of *Hae*II and *Rsa*I and the addition of *Ava*I and *Hpa*I; *Dra*I gave poor results in some accessions of the present study and was not scored. Membranes were probed with 12 *Pst*I and two *Sal*I clones of *Petunia* (Sytsma and Gottlieb

TABLE 1. Accessions examined for cpDNA variation, arranged alphabetically and then geographically from the northwest to the southeast. Three accessions of *S. cardiophyllum* (51–53) were not included in the analysis because of polymorphism in pooled leaf samples. ¹Taxon abbreviations follow Hawkes (1990): blb blb = *S. bulbocastanum* subsp. *bulbocastanum*, blb dph = *S. bulbocastanum* subsp. *dolichophyllum*, blb ptt = *S. bulbocastanum* subsp. *partitum*, cph cph = *S. cardiophyllum* subsp. *cardiophyllum*, cph ehr = *S. cardiophyllum* subsp. *ehrenbergii*, cph lcl = *S. cardiophyllum* subsp. *lanceolatum*, pnt = *S. pinnatisectum*, pls = *S. palustre*. ²United States Department of Agriculture Plant Introduction Numbers. Recent collections by Rodríguez et al. (1995) have not yet been assigned PI numbers.

Taxon ¹	PI ²	Collector	Locality
1. blb blb	275187	Hawkes 1584	Mexico. Michoacán: near Zacapu, at km 400 from México City on the road to Guadalajara; 19°50'N, 101°43'W.
2. blb blb	347757	Tarn 153	Mexico. Michoacán: México City-Morelia road, Hwy 15, at junction with road to Maravatio; 2,060 m; 19°31'N, 100°15'W.
3. blb blb	275185	Hawkes 1582	Mexico. Distrito Federal: Pedregal de San Angel, Ciudad Universitaria; 19°56'N, 101°22'W.
4. blb blb	275186	Hawkes 1583	Mexico. Distrito Federal: Pedregal de San Angel, Ciudad Universitaria; 19°56'N, 101°22'W.
5. blb blb	275197	Hawkes 1594	Mexico. Distrito Federal: Cuicuilco, Pedregal; 2,200 m; 15°55'N, 99°53'W.
6. blb blb	275188	Hawkes 1585	Mexico. México: road from Amecameca to Chalco, Tlalmanalco; 2,400 m; 19°13'N, 98°48'W.
7. blb blb	275189	Hawkes 1586	Mexico. México: road from Amecameca to Chalco, Tlalmanalco; 19°11'N, 98°13'W.
8. blb blb	275198	Hawkes 1595	Mexico. México: Cerro de Cocotitlán; 19°43'N, 99°47'W.
9. blb blb	275199	Hawkes 1596	Mexico. México: near Texcoco, Chapingo, Casas Antiguas; 2,200 m; 19°29'N, 98°54'W.
10. blb blb		Rodríguez et al. 2546	Mexico. México: municipality of Texcoco, Tetzcuiztingo mountain, right next to San Nicolás Tlamaca; 2,400 m; 19°32'N, 99°50'W.
11. blb blb	275192	Hawkes 1589	Mexico. Tlaxcala, km 8 on the road from Tlaxcala to Puebla; 19°11'N, 98°13'W.
12. blb blb	545711	Tarn et al. 149	Mexico. Guerrero: 8 km towards Huitziltepec from El Platanal on Hwy 95 from Chilpancingo to México City; 1,500 m; 17°33'N, 99°30'W.
13. blb blb	275194	Hawkes 1591	Mexico. Oaxaca: ruins of Monte Albán; 1,950 m; 17°02'N, 96°46'W.
14. blb blb	275195	Hawkes 1592	Mexico. Oaxaca: ruins of Monte Albán; 1,950 m; 17°02'N, 96°46'W.
15. blb blb	275196	Hawkes 1593	Mexico. Oaxaca: road from Oaxaca to Valle Nacional; 1,590 m; 17°30'N, 96°27'W.
16. blb blb	283096	Hawkes 1719	Mexico. Oaxaca: Sierra de las Mixtecas, near Siete Cabrillas, 475 km from México City on the road to Oaxaca; 17°30'N, 96°52'W.
17. blb blb	498011	Tarn et al. 173	Mexico. Oaxaca: ruins of Monte Albán near Oaxaca City; 2,000 m; 17°02'N, 96°46'W.
18. blb blb		Rodríguez et al. 2509	Mexico. Oaxaca: municipality of San Felipe del Agua, foot-hills of San Felipe mountains, 500 m N of the town; 1,830 m; 17°07'N, 96°42'W.
19. blb blb		Rodríguez et al. 2518	Mexico. Oaxaca: municipality of San Felipe del Agua, road to San Felipe del Agua mountains; 1,670 m; 17°07'N, 96°42'W.
20. blb blb		Rodríguez et al. 2523	Mexico. Oaxaca: Oaxaca-Monte Albán road, just at the junction to San Juanito; 1,820 m; 17°02'N, 96°46'W.
21. blb dph		Rodríguez et al. 2580	Mexico. Jalisco: Casa Blanca, along the El Tigre streamside, municipality of Poncitlán; 1,600 m; 20°28'N, 103°03'W.
22. blb dph		Rodríguez et al. 2581	Mexico. Jalisco: Rancho Villa Quieta, road from Cuquío to Yahualica, municipality of Cuquío; 1,920 m; 21°01'N, 102°59'W.
23. blb dph	255516	Graham 300B	Mexico. Jalisco: Ciudad Guzmán.

TABLE 1. Continued.

Taxon ¹	PI ²	Collector	Locality
24. blb dph	498224	<i>Ochoa 14162</i>	Mexico. Michoacán: Las Palmitas; 1,825 m; 21°13'N, 99°02'W.
25. blb dph	545752	<i>Tarn et al. 244</i>	Mexico. México: east of Coatepec Harinas, between San Francisco and Porfirio Díaz; 2,450 m; 18°51'N, 99°43'W.
26. blb dph		<i>Rodríguez et al. 2548</i>	Mexico. Morelos: La Pera, Hwy from México City to Cuernavaca; 2,225 m; 19°01'N, 99°10'W.
27. blb ptt	558379	<i>Spooner et al. 4224</i>	Mexico. Chiapas: 9.4 km S of route 190 (from near Teopisca), to Las Rosas; 1,720 m; 16°10'N, 92°12'W.
28. blb ptt	275200	<i>Hawkes 1796</i>	Guatemala. Quetzaltenango: road from Huehuetenango to Quetzaltenango, 5 km S of Malacatancito; 1,800 m; 15°10'N, 91°31'W.
29. cph cph	283062	<i>Graham s.n.</i>	Mexico. México.
30. cph cph		<i>Rodríguez et al. 2551</i>	Mexico. Morelos: Texcal, road from Cuernavaca to Tepoztlán; 1,750 m; 18°58'N, 99°10'W.
31. cph cph	347759	<i>Tarn 241D</i>	Mexico. Puebla: left side of Tehuacán-Huajuapán de León road on the Puebla side; 2,060 m; 18°37'N, 97°35'W.
32. cph cph		<i>Rodríguez et al. 2529</i>	Mexico. Puebla: San Antonio del Río, municipality of San Miguel Ixitlán, on the Huajuapán de León-Tehuacán road; 1,930 m; 18°00'N, 97°42'W.
33. cph cph		<i>Rodríguez et al. 2541</i>	Mexico. Puebla: Cañada de Rojas, road from Tehuacán to Orizaba; 2,150 m; 18°40'N, 97°22'W.
34. cph ehr	279272	<i>Hawkes 1458</i>	Mexico. Aguascalientes: 7 mi from Aguascalientes on the road to Loreto, 1.25 mi from the main Aguascalientes to Zacatecas Hwy; 1,900 m; 21°53'N, 102°18'W.
35. cph ehr		<i>Rodríguez et al. 2566</i>	Mexico. San Luis Potosí: Villa de Arriaga, road from San Luis Potosí to Ojuelos; 2,240 m; 21°54'N, 101°24'W.
36. cph ehr		<i>Rodríguez et al. 2579</i>	Mexico. Jalisco: municipality of Valle de Guadalupe, near town and along the road; 1,820 m; 21°00'N, 102°37'W.
37. cph ehr		<i>Rodríguez et al. 2584</i>	Mexico. Jalisco: experimental field around the College of Agriculture, University of Guadalajara, municipality of Zapopan; 1,500 m; 20°43'N, 103°32'W.
38. cph ehr		<i>Rodríguez et al. 2586</i>	Mexico. Jalisco: Matanzas, near by the cementary, municipality of Ojuelos; 2,190 m; 21°37'N, 101°38'W.
39. cph ehr		<i>Rodríguez 2588</i>	Mexico. Jalisco: road from Cd. Guzmán to El Grullo, just at the junction to El Fresno, municipality of Cd. Guzmán; 1,770 m; 19°37'N, 103°32'W.
40. cph ehr		<i>Rodríguez et al. 2564</i>	Mexico. Guanajuato: La Purísima, municipality of San Diego de la Unión, road from Querétaro City to San Luis Potosí; 2,060 m; 21°23'N, 100°41'W.
41. cph ehr	184762	<i>Hawkes 1086</i>	Mexico. Querétaro: San Juan del Río, SW of the town; 2,050 m; 20°40'N, 99°30'W.
42. cph ehr	275216	<i>Hawkes 1421</i>	Mexico. Querétaro: San Juan del Río-Querétaro road, 17 mi before Bernal, Cerro Galeras, E of the road; 2,050 m; 20°38'N, 99°56'W.
43. cph ehr	275213	<i>Hawkes 1428</i>	Mexico. Querétaro: 3 km SSE of town; 1,900 m; 20°36'N, 100°23'W.
44. cph ehr	275214	<i>Hawkes 1429</i>	Mexico. Querétaro: Santa Rosa, road from Querétaro to San Luis Potosí; 2,050 m; 20°44'N, 100°27'W.
45. cph ehr		<i>Rodríguez et al. 2497</i>	Mexico. Querétaro: municipality of Querétaro, road Querétaro-México City, just at the intersection to El Rosario, E side of Monín Indian monument; 2,000 m; 20°34'N, 100°19'W.
46. cph ehr		<i>Rodríguez et al. 2533</i>	Mexico. Puebla: Santiago Acatepec, municipality of Caltepec, on the side of the road from Huajuapán de León to Tehuacán; 2,050 m; 18°13'N, 97°35'W.
47. cph lcl		<i>Rodríguez et al. 2529</i>	Mexico. Puebla: San Antonio del Río, municipality of San Miguel Ixitlán, on the Huajuapán de León-Tehuacán road; 1,930 m; 18°00'N, 97°42'W.

TABLE 1. Continued.

Taxon ¹	PI ²	Collector	Locality
48. cph lcl		Rodríguez et al. 2534	Mexico. Puebla: road from Tehuacán towards Orizaba, just at the junction to Miahuatlán; 1,870 m; 18°32'N, 97°25'W.
49. pnt	275234	Hawkes 1456	Mexico. Jalisco: road from León to Aguascalientes at 15 mi from León; 1,950 m; 21°30'N, 101°59'W.
50. pls	218228	Erwin Baur Sortiment Genebank, Germany	Argentina or Chile; specific locality unknown.
51. cph cph		Rodríguez et al. 2570	Mexico. Jalisco: Matanzas, municipality of Ojuelos, near by the cemetery; 2,190 m; 21°37'N, 101°38'W.
52. cph ehr		Rodríguez et al. 2567	Mexico. Zacatecas: ca. 1 km N of Ojuelos; 2,212 m; 21°52'N, 101°34'W.
53. cph ehr	545753	Tarn et al. 211	Mexico. San Luis Potosí: Hwy 49 at 67 km W of Zacatecas, then 1 km N along the track to Villa de Ramos; 2,160 m; 22°39'N, 101°57'W.

1986), and five small single-copy region clones of *Nicotiana* L. (Olmstead and Palmer 1992), covering the entire chloroplast genome. Clones were radiolabelled by ³²P-dCTP by the oligo-labelling method of Feinberg and Vogelstein (1984).

Phylogenetic reconstructions were performed with PAUP, version 3.1.1 (Swofford 1993). The data were analyzed using Wagner parsimony (Farris 1970), which gives equal weight to site gains and site losses. The most parsimonious trees were sought using BRANCH AND BOUND, COLLAPSE, and MULPARS. Optimal reconstruction was performed using ACCTRAN, which favors reversals over parallelisms. Additionally, the character-state weighting method of Albert et al. (1992) was used with weights of 1.1 and 1.3 given to site gains to account for the greater relative probability of restriction site gains at this taxonomic level. Bootstrap values (Felsenstein 1985) on resulting branches were obtained from 5,000 replicates using Wagner parsimony, random order entry, and NNI. Decay analyses were conducted with inverse constraint searches in PAUP.

RESULTS

A total of 62 restriction site variants were identified (Tables 2,3). *Ava*I and *Xho*I gave three and one new variants, respectively, relative to Spooner and Sytsma (1992; variants 1–3, 59; Table 2, Fig. 5). One accession of *S. cardiophyllum* subsp. *cardiophyllum* (51; Table 1), and two accessions of subsp. *ehrenbergii* (52, 53) were excluded in the final analysis because they exhibited within-sample polymorphism in the P10 region, located in the large single copy region.

Wagner parsimony analysis produced two most parsimonious 72-step trees with a consistency index of 0.86 (without autapomorphies) and a retention index of 0.98. These two trees differed only in the topology of *S. cardiophyllum* accessions 32 (*S. cardiophyllum* subsp. *cardiophyllum*), and 47 and 48 (*S. cardiophyllum* subsp. *lanceolatum*). Weighted parsimony, with weights of 1.1 and 1.3 in favor of gains over losses, generated a single most-parsimonious tree identical in topology to one of the two Wagner trees.

The weighted parsimony tree (Fig. 5) supports two main clades, exclusive of *S. palustre*:

1. All accessions of *S. bulbocastanum* and *S. cardiophyllum* exclusive of *S. cardiophyllum* subsp. *ehrenbergii*.
2. All accessions of *S. cardiophyllum* subsp. *ehrenbergii*, *S. brachistotrichum*, *S. pinnatisectum*, and *S. stenophyllidium*. A comparison of restriction site variants of this tree to those of Spooner and Sytsma (1992; Fig. 1) shows 12 of the 13 accessions of subsp. *ehrenbergii* to fall in the clade containing *S. brachistotrichum* and *S. stenophyllidium*, and 1 of the 13 (accession 34) to be removed from this clade by a single restriction site variant. The accession of *S. pinnatisectum* examined here is indistinguishable from *S. pinnatisectum* 1 of Spooner and Sytsma (1992) (Fig. 1).

DISCUSSION

Alternative Hypotheses for the Origin and Relationships of *S. cardiophyllum* subsp. *ehrenbergii*. Relative to the cpDNA study of Spooner and Sytsma (1992) (Fig. 1) our results: 1) support the sister group relationship of *S. bulbocastanum* and *S.*

TABLE 2. Chloroplast DNA restriction site variants in *S. brachistotrichum*, *S. bulbocastanum*, *S. cardiophyllum*, *S. pinnatisectum*, *S. palustre*, and *S. stenophyllidium*. The variants are listed with the apomorphic state first, followed by the plesiomorphic state (relative to *S. palustre*). A double arrow indicates that a variant could not be polarized because *S. palustre* differed from all other species or data were not available for this species. Parentheses indicate where small fragments were hypothesized to exist because length variants were not observed with other enzymes. See Table 3 for character states.

Restriction site variant number	Enzyme	Region	Size (kb)
1	<i>Ava</i> I	P10	3.0 + 0.5 = 3.5
2	<i>Ava</i> I	P10	1.7 ↔ 1.6 + (0.1)
3	<i>Ava</i> I	P3	6.8 = 5.5 + 1.3
4	<i>Bam</i> HI	P10	3.4 ↔ 2.8 + 0.6
5	<i>Bam</i> HI	P10	2.5 + (0.2) = 2.7
6	<i>Bam</i> HI	P3	9.9 = 6.0 + 3.9
7	<i>Bam</i> HI	P12/P14	5.9 = 3.5 + 2.4
8	<i>Ban</i> II	P10	1.8 + 1.0 = 2.8
9	<i>Ban</i> II	P6	1.4 + 1.4 = 2.8
10	<i>Bcl</i> I	P10	0.7 ↔ 0.5 + (0.2)
11	<i>Bcl</i> I	P10	0.6 † (0.1) = 0.7
12	<i>Bcl</i> I	P6	8.1 ↔ 6.2 + 1.9
13	<i>Bcl</i> I	P16/S6	4.0 + (0.4) = 4.4
14	<i>Bcl</i> I	T33/T34/T35	4.6 ↔ 3.6 + 1.0
15	<i>Bcl</i> I	T38	1.4 + (0.8) = 2.2
16	<i>Bgl</i> II	S8	3.3 ↔ 2.3 + 1.0
17	<i>Bgl</i> II	P12	0.7 = 0.4 + 0.3
18	<i>Bst</i> NI	P6	1.6 ↔ 1.4 + (0.2)
19	<i>Bst</i> NI	S8	4.6 = 3.3 + 1.3
20	<i>Bst</i> NI	P16/S6	2.7 + 1.2 = 3.9
21	<i>Cla</i> I	P6	8.9 ↔ 5.3 + 3.6
22	<i>Cla</i> I	P3	6.3 = 5.3 + 1.0
23	<i>Cla</i> I	T36/T37	5.8 + 1.3 = 7.1
24	<i>Cla</i> I	T40	2.4 = 1.5 + 0.9
25	<i>Eco</i> O 109	P10/P19	6.3 + 4.9 = 11.2
26	<i>Eco</i> O 109	P6	2.9 = 2.1 + 0.8
27	<i>Eco</i> O 109	P3	6.1 ↔ 5.3 + 0.8
28	<i>Eco</i> RI	P10/P8	2.0 ↔ 1.7 + 0.3
29	<i>Eco</i> RI	P6	0.7 = 0.5 + (0.2)
30	<i>Eco</i> RI	P3	3.6 = 3.4 + (0.2)
31	<i>Eco</i> RI	P16/S6	1.6 + (0.1) = 1.7
32	<i>Eco</i> RI	S8	1.6 + 0.6 = 2.2
33	<i>Eco</i> RI	P12/P14	1.2 + 1.0 = 2.2
34	<i>Eco</i> RI	T36/T37	2.5 = 1.7 + 0.8
35	<i>Eco</i> RI	T40	2.8 = 2.3 + (0.5)
36	<i>Eco</i> RV	T37/T38	11.0 = 8.0 + 3.0
37	<i>Hinc</i> II	P6/P8	8.0 + 0.4 = 8.4
38	<i>Hinc</i> II	P3	3.5 ↔ 2.7 + 0.8
39	<i>Hinc</i> II	T37	3.6 = 2.1 + 1.5
40	<i>Hinc</i> II	T38	2.2 = 1.6 + (0.6)
41	<i>Hind</i> III	P3	4.0 ↔ 2.9 + 1.1
42	<i>Hind</i> III	T36/T37	4.4 + 2.2 = 6.6
43	<i>Hpa</i> II	P10	0.8 = 0.6 + (0.2)
44	<i>Hpa</i> II	P6	1.5 + (0.4) = 1.9
45	<i>Hpa</i> II	P16/S6	2.2 ↔ 2.0 + (0.2)
46	<i>Hpa</i> II	T39/T40	5.9 = 3.3 + 2.6

TABLE 2. Continued.

Restriction site variant number	Enzyme	Region	Size (kb)
47	<i>Hpa</i> II	T39/T40	1.4 + 1.2 = 2.6
48	<i>Hph</i> I	P10	9.9 = 6.0 + 3.9
49	<i>Hph</i> I	P10	3.0 + 3.0 = 6.0
50	<i>Hph</i> I	P16/S6	1.0 + 0.7 = 1.7
51	<i>Hph</i> I	T39/T40	1.7 + 1.5 = 3.2
52	<i>Nci</i> I	P10	5.5 = 4.9 + 0.6
53	<i>Nci</i> I	P6	2.7 + (0.4) = 3.1
54	<i>Nci</i> I	P6	3.2 + (0.1) = 3.3
55	<i>Nci</i> I	S8	1.7 + 0.4 = 2.1
56	<i>Nci</i> I	T39/T40	6.9 = 4.3 + 2.6
57	<i>Nsi</i> I	P10/P8	18.6 = 12.0 + 6.6
58	<i>Nsi</i> I	P10	8.4 = 6.6 + 1.8
59	<i>Sst</i> I	P10/P8	5.1 + 0.9 = 6.0
60	<i>Sst</i> I	P6/P3	9.0 + 9.0 = 18.0
61	<i>Sst</i> I	S6/S8	6.8 + 3.7 = 10.5
62	<i>Xho</i> I	P3	10.6 ↔ 5.5 + 5.1

cardiophyllum, except for *S. cardiophyllum* subsp. *ehrenbergii*, and 2) place all accessions of subsp. *ehrenbergii* in the clade containing the other Mexican and Central American diploid species exclusive of *S. verrucosum* Schtdl. (Fig. 1), with 12 of these 13 accessions on the clade containing *S. brachistotrichum* and *S. stenophyllidium* (ser. *Pinnatisecta*). The results prompt a reevaluation of the criteria that Correll (1962) and Hawkes (1990) used for allying subsp. *ehrenbergii* with *S. cardiophyllum* rather than with *S. brachistotrichum* or *S. stenophyllidium*. Subspecies *ehrenbergii* shares with other *S. cardiophyllum* subspecies leaves with 2–4 pairs of lateral leaflets, plants with coarse and sparse pubescence erect or pointed towards the apex of the leaflet, and petiolulate leaflets, in contrast to the similar species *S. brachistotrichum* and *S. stenophyllidium* that have leaves with 3–5 pairs of lateral leaflets, pilose pubescence pointing towards the base of the leaflet, and sessile or decurrent leaflets. Subspecies *ehrenbergii* shares with all members of ser. *Pinnatisecta* corollas white or tinged with purple, in contrast to the yellowish-white corollas of *S. cardiophyllum* (Correll 1962; Hawkes 1990). Correll (1962) noted a similarity of subsp. *ehrenbergii* to *S. brachistotrichum* and suggested that subsp. *ehrenbergii* was a “connecting link” between the other subspecies of *S. cardiophyllum* and ser. *Pinnatisecta*. However, he was dissuaded from allying subsp. *ehrenbergii* to *S. brachistotrichum* because of geographical isolation. *Solanum brachistotrichum* is distributed in northern Mexico in the states of Chihuahua, Sonora, Durango, Zacatecas, and Jalisco, and *S. stenophyllidium*, in west central Mexico in the states of

TABLE 3. Data matrix of 62 restriction site variants for the 50 examined accessions, with additional data of *S. brachistotrichum* and *S. stenophyllidium* from Spooner and Sytsma (1992). The states 0, 1, and 9 represent site absence, site presence, and missing data, respectively. The accession numbers are from the first column of Table 2.

<i>Solanum bulbocastanum</i> subsp. <i>bulbocastanum</i> 1, 3-5, 7, 8, 10	0 0 0 0 1 0 0 1 1 1 0 1 0 0 1 0 1 1 1 1 0 1 0 1 0 1 0 1 1 0 0 1 0 1 0 1 0 0 1 1 0 0 1 1 0 0 0 1 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0
<i>S. bulbocastanum</i> subsp. <i>bulbocastanum</i> 2, 6, 9, 12, 13-20; <i>S. bulbocastanum</i> subsp. <i>dolichophyllum</i> 21-24; <i>S. bulbocastanum</i> subsp. <i>partitum</i> 27, 28	0 0 0 0 1 0 0 1 1 1 0 1 0 0 1 0 1 1 1 1 0 1 0 1 0 1 0 1 1 0 0 1 0 1 1 1 0 0 1 1 0 0 1 1 0 0 0 1 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0
<i>S. bulbocastanum</i> subsp. <i>bulbocastanum</i> 11	1 0 0 0 1 0 0 1 1 1 0 1 0 0 1 0 1 1 1 1 0 1 0 1 0 1 0 1 1 0 0 1 0 1 1 1 0 0 1 1 0 0 1 1 0 0 0 1 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0
<i>S. bulbocastanum</i> subsp. <i>dolichophyllum</i> 25	0 0 0 0 1 0 0 1 1 1 0 1 0 0 1 0 1 1 1 1 0 1 0 1 0 1 0 1 1 0 0 1 0 1 1 1 0 0 1 1 0 0 1 1 0 0 0 1 0 0 0 1 1 0 0 0 1 1 0 0 0 0 1 1 1 0 0 0
<i>S. cardiophyllum</i> subsp. <i>cardiophyllum</i> 29, 31	0 0 1 0 0 0 1 1 0 1 0 1 1 0 1 0 1 1 1 0 0 1 1 1 1 0 1 1 1 1 0 0 0 1 0 1 0 1 0 1 0 0 0 1 1 0 1 0 1 1 1 0 1 1 0 0 1 1 1 1 0 0 1 1 1 1 0 0 1
<i>S. cardiophyllum</i> subsp. <i>cardiophyllum</i> 30	0 0 1 0 0 0 1 1 0 1 0 1 1 0 1 0 1 1 1 0 0 1 1 0 1 1 0 1 1 1 1 0 0 0 1 0 1 0 1 0 1 0 0 0 1 1 0 1 0 1 1 1 0 1 1 1 0 0 1 1 1 1 0 0 1 1 1 1 0 0 1
<i>S. cardiophyllum</i> subsp. <i>cardiophyllum</i> 32	0 0 1 0 0 0 1 1 0 1 0 1 1 0 1 0 1 1 1 0 0 1 1 1 1 1 0 1 1 1 0 0 0 0 1 0 1 0 0 0 0 0 1 1 0 1 0 1 0 1 0 1 1 0 0 1 1 1 1 0 0 1 1 1 1 0 0 1
<i>S. cardiophyllum</i> subsp. <i>cardiophyllum</i> 33	0 0 1 0 0 0 1 1 0 1 0 1 1 0 1 0 1 1 1 0 0 0 1 1 1 1 1 0 1 1 1 1 0 0 0 1 0 1 0 1 0 1 0 0 0 1 1 0 1 0 1 1 1 0 1 1 0 0 1 1 1 1 0 0 1 1 1 1 0 0 1
<i>S. cardiophyllum</i> subsp. <i>ehrenbergii</i> 34	1 0 1 0 1 0 1 0 0 1 0 1 1 0 1 0 0 1 0 0 0 0 0 1 0 0 0 1 0 1 0 0 0 1 1 1 0 0 1 1 0 1 0 0 0 1 1 1 0 0 1 0 0 0 1 1 1 1 0 0 1 1 1 1 0 0 1 1
<i>S. cardiophyllum</i> subsp. <i>ehrenbergii</i> 35-37, 39	1 0 1 0 1 0 1 0 0 1 1 1 1 0 1 0 0 1 0 0 0 0 0 1 0 0 0 1 0 1 0 0 1 1 1 1 0 0 1 1 0 1 0 0 0 1 1 1 0 0 1 0 0 0 1 1 1 1 0 0 1 0 0 0 1 1 1 1 0 0 1 1
<i>S. cardiophyllum</i> subsp. <i>ehrenbergii</i> 38	1 0 1 0 1 0 1 0 0 1 0 1 1 0 1 0 0 1 0 0 0 0 0 1 0 0 0 1 0 1 0 0 1 1 1 1 0 0 1 1 0 1 0 0 0 1 1 1 0 0 0 1 0 0 0 1 0 0 0 1 1 1 1 0 0 1 1
<i>S. cardiophyllum</i> subsp. <i>ehrenbergii</i> 40, 41, 43-46	1 0 1 0 1 0 1 0 0 1 0 1 1 0 1 0 0 1 0 0 0 0 0 1 0 0 0 1 0 1 0 0 1 1 1 1 0 0 1 1 0 1 0 0 0 1 1 1 0 0 1 0 0 0 1 1 1 0 0 1 0 0 0 1 1 1 1 0 0 1 1
<i>S. cardiophyllum</i> subsp. <i>ehrenbergii</i> 42	1 0 1 0 1 0 1 0 0 1 0 1 1 0 0 0 0 1 0 0 0 0 0 1 0 0 0 1 0 1 0 0 1 1 1 1 0 0 1 1 0 1 0 0 0 1 1 1 0 0 1 0 0 0 1 1 1 0 0 1 0 0 0 1 1 1 1 0 0 1 1
<i>S. cardiophyllum</i> subsp. <i>lanceolatum</i> 47	0 0 1 0 0 0 1 0 0 1 0 1 1 0 1 0 1 1 0 0 0 0 0 1 1 1 0 1 0 1 0 0 1 1 1 0 1 0 0 0 0 0 1 1 0 1 0 1 0 1 0 1 1 0 0 1 1 1 1 0 0 1 1 1 1 0 0 1
<i>S. cardiophyllum</i> subsp. <i>lanceolatum</i> 48	0 0 1 0 0 0 1 1 0 1 0 1 1 0 1 0 1 1 1 0 0 1 1 1 1 1 0 1 1 1 0 0 0 0 1 0 1 0 1 0 0 0 1 1 0 1 0 1 0 1 0 1 1 0 0 1 1 1 0 0 1 1 1 1 0 0 1
<i>S. pinnatisectum</i> 49	1 0 1 0 1 0 1 0 0 1 0 1 1 0 1 0 0 1 0 0 0 0 0 1 0 0 0 1 0 1 0 0 0 1 1 1 0 0 1 1 0 1 0 0 0 1 1 1 0 0 1 0 0 0 1 1 1 0 0 1 0 0 0 1 1 1 0 0 0 1 0
<i>S. palustre</i> 50	0 1 1 1 1 1 1 0 0 0 0 0 0 1 1 1 1 0 1 0 1 1 0 0 0 1 1 0 1 1 0 0 0 1 1 1 0 1 1 1 1 0 1 0 1 1 0 1 0 0 0 1 0 0 0 1 1 1 0 0 0 9
<i>S. brachistotrichum</i> PI 255527, <i>S. stenophyllidium</i> PI 518629	1 0 1 0 1 0 1 0 0 1 0 1 1 0 1 0 0 1 0 0 0 0 0 1 0 0 0 1 0 1 0 0 1 1 1 1 0 0 1 1 0 1 0 0 0 1 1 1 0 0 1 0 0 0 1 1 1 0 0 1 0 0 0 1 1 1 1 0 0 1 1

Aguascalientes and Jalisco, with both barely overlapping the northwestern end of the range of subsp. *ehrenbergii* (Correll 1962; Hawkes 1966, 1990; Rodríguez 1991).

Chloroplast DNA may be misleading in indicat-

ing the sister group relationships of subsp. *ehrenbergii* to *S. brachistotrichum* or *S. stenophyllidium*. Rather, this subspecies may have been formed by hybridization of *S. cardiophyllum* subsp. *cardiophyllum* or subsp. *lanceolatum* as paternal parent, with

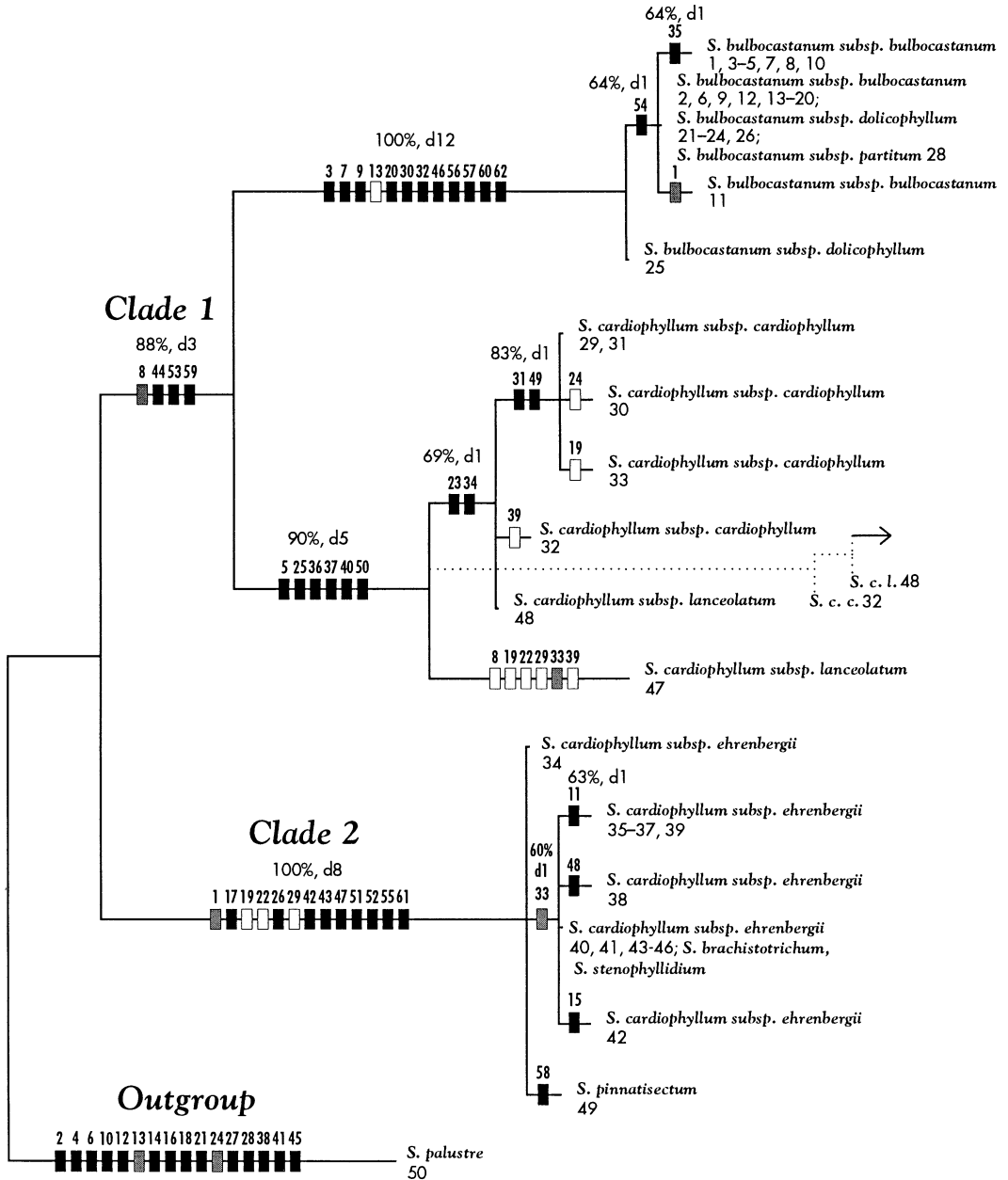


FIG. 5. One of two most-parsimonious 72-step Wagner trees (as a phylogram) of the cpDNA data, and the single most-parsimonious tree (1.1 and 1.3 weights of site gains over site losses), with overlaid bootstrap and decay values and enzyme site variants supporting each branch. Characters are optimized on the tree using accelerated transformation option. Gray and open boxes represent homoplastic gains and losses, respectively. The difference in the topology of the second most-parsimonious Wagner involves only two accessions of *Solanum cardiophyllum*, and is indicated by dashed lines.

some other member of ser. *Pinnatisecta* as maternal parent, with subsequent backcrossing to the paternal parent to obtain the chloroplast of the maternal donor. Chloroplast DNA evidence suggests *S.*

brachistotrichum or *S. stenophyllidium* as maternal parent, but the relatively low cpDNA divergence of other species in ser. *Pinnatisecta* (Spooner and Sytsma, 1992; Fig. 1) and wide fertility makes all

species in this series possibilities. Geographical evidence suggests *S. pinnatisectum* as a possible maternal parent, because *S. cardiophyllum* and *S. pinnatisectum* have widely overlapping ranges from central Mexico in Jalisco south to Michoacán, unlike *S. brachistotrichum* and *S. stenophyllidium*, distributed on the northwestern edge of subsp. *ehrenbergii* range to much farther north, nearly to the border with the United States (Correll 1962; Hawkes 1966, 1990). *Solanum cardiophyllum* and *S. pinnatisectum* can be crossed artificially to form fertile hybrids, and subsp. *ehrenbergii* is a weedy taxon growing in cultivated fields with *S. pinnatisectum* (pers. observation), unlike the other subspecies of *S. cardiophyllum* growing in less disturbed habitats.

Subspecies Circumscriptions. The cpDNA data show *S. cardiophyllum* subsp. *ehrenbergii* to be part of a distinct clade from both subsp. *cardiophyllum* and subsp. *lanceolatum*, but fail to partition the other subspecies of *S. bulbocastanum* and *S. cardiophyllum* into clades. The lack of support for subspecies may indicate that they are invalidly recognized, or that cpDNA restriction site data are too conservative to distinguish them. Alternatively, the cpDNA polymorphisms may be the result of lineage sorting or hybridization. The resolution of subspecies of *S. bulbocastanum* and *S. cardiophyllum* will require less conservative molecular markers, and a comprehensive morphological study with other members of ser. *Pinnatisecta*.

Intraspecific cpDNA Variation. Intraspecific cpDNA variation is common among many angiosperm groups that have been intensively studied (Harris and Ingram 1991; Soltis et al. 1992), including sect. *Petota* (Hosaka and Hanneman 1988a,b; Hosaka 1995). These data show intraspecific cpDNA polymorphisms in a wide range of accessions of *S. bulbocastanum* and *S. cardiophyllum*. This study also found cpDNA polymorphism confined to the P10 probe region in multiple pooled individuals of three accessions (Table 1; 51, *S. cardiophyllum* subsp. *cardiophyllum*; 52, 53, *S. cardiophyllum* subsp. *ehrenbergii*); whether these samples represent polymorphism within individuals (heteroplasmy) is being examined further.

These unexpected cpDNA topological results were obtained through intensive intraspecific sampling. Hybridization and introgression are believed to be widespread in sect. *Petota* (Ugent 1970; Grun 1990; Hawkes 1990) and may be a cause of some of the polymorphism found in this study and elsewhere in the group. Rieseberg and Soltis (1991) and Rieseberg (1995) showed that the possibility of widespread introgression and hybridization in

plants necessitates such intensive sampling for molecular studies. This study shows relatively moderate cpDNA variation in *S. bulbocastanum* and *S. cardiophyllum*, but with extensive cpDNA divergence in *S. cardiophyllum* subsp. *ehrenbergii* that places it in a separate clade. The resolution of the origins and relationships of the subspecies of *S. bulbocastanum* and *S. cardiophyllum* will require additional morphological and molecular studies using biparentally inherited markers to test the concordance of cpDNA gene trees to species trees (e.g., Doyle 1992). Conclusions of hybrid origins based on morphological data may be complicated by the fact that hybrids are not always intermediate in appearance relative to their parents (Rieseberg 1995). Such comparative data with many accessions are needed throughout the taxonomically confusing sect. *Petota* to reexamine species boundaries and relationships and assess the extent and significance of hybridization.

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