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MORPHOLOGICAL CHARACTERIZATION AND RELATIONSHIPS OF WILD TOMATOES (*SOLANUM* L. SECT. *LYCOPERSICON*)

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Abstract. Wild tomatoes (*Solanum* L. sect. *Lycopersicon* (Mill.) Wettst.) are native to western South America. Different classifications have been based on morphological or biological species concepts. Molecular data from mitochondrial DNA restriction sites, nuclear and chloroplast DNA restriction length fragment polymorphisms, and most recently gene sequences of the single-copy nuclear GBSSI or *waxy* gene, also have been used to examine species relationships. This study is a companion to the GBSSI gene sequence study of the same accessions. It provides the first explicit use of morphological data to examine distinctness and relationships of all 10 wild tomato species (including the newly described *S. galapagense*), with a concentration on accessions of the most widespread and variable species, *S. peruvianum*. Phenetic and cladistic analyses largely support the nine species outlined by the latest treatment by C. Rick, but demonstrate the distinct nature of the northern and southern Peruvian populations of *S. peruvianum*, and suggest that they may represent distinct species.

Key words: phylogeny, *Solanum* sect. *Lycopersicon,* tomato.

Wild tomatoes are mainly characterized by anthers with sterile appendages, laterally connivent forming a flask-shaped cone. All species are native to western South America and distributed from central Ecuador, through Peru to northern Chile, and in the Galápagos Islands, where two endemic species, *S. cheesmaniae* and *S. galapagense,* grow (Darwin et al., 2003). Wild tomato species grow in a variety of habitats, from near sea level along the arid Pacific coast to over 3300 m in the numerous valleys of the western side of the Andes (Rick, 1973; Taylor, 1986). The wild ancestor of cultivated tomatoes, *S. lycopersicum*, is more widespread and perhaps more recently distributed into Mexico, Colombia, Bolivia, and other South American countries (Rick & Holle, 1990). Wild species provide a wealth of useful genetic traits to improve cultivated tomatoes. The traditional breeding for pure lines in the crop has contributed to its narrow genetic base (Stevens & Rick, 1986). All wild tomato species are diploids ($2n = 2x = 24$) and can be crossed (but sometimes with difficulty) to the cultivated tomato. They are of great use in breeding programs as sources of disease resistance and agronomic traits (Esquinas Alcazar, 1981; Laterrot, 1989; Rick, 1982a, 1986a, 1987; Rick et al., 1987; Stevens & Rick, 1986). The tomato also serves as a model organism for genetic studies (Tanksley & McCouch, 1997; Hay et al., 2004).

Wild tomatoes exhibit great differences in morphological characters, mating systems, and habitat preferences. In their natural habitats, wild tomatoes probably behave as annuals because frost or drought kills the plants after the first growing season, but they can be biennial or perennial herbs (Müller, 1940). All tomato species are pubescent, and trichome types and density are valuable characters to distinguish species. Stems are slender and herbaceous throughout, but also can undergo secondary growth at the base. Habits vary from erect to procumbent to decumbent. Leaves are alternate, imparipinnate to bipinnate, with 2 to 6 opposite or sub-opposite pairs of petiolate or subsessile leaflets, and sometimes with additional small sessile or subsessile interjected leaflets. Leaf dissection distinguishes subspecies or varieties of *S. peruvianum*. The basic inflorescence includes monochasial, dichotomous, and polychotomous cymes (Luckwill, 1943). Some wild tomato species develop inflorescence bracts and pseudostipules at the base of the leaf petiole. Flowers are typically yellow, the calyx is divided in five lobes that are accrescent about the fruits, and the corolla is stellate or stellate-rotate. The five anthers have an elongated sterile appendage at the apex and are laterally connivent forming a flask-shaped cone, except in *S. pennellii*. Fruits are fleshy berries with two or rarely more locules, variable in size, shape, color, and pubescence.

Müller (1940) and Luckwill (1943) produced the two most recent and complete taxonomic treatments of wild tomatoes based on morphological concepts, and treated them under *Lycopersicon* (Fig. 1). Müller (1940) divided the genus into two subgenera: (1) *Eulycopersicon* C. H. Müll., with two species possessing glabrous, and red- to orange- to yellow-colored fruits; flat and silky pubescent seeds; bractless inflorescences; and leaves without pseudostipules; and (2) *Eriopersicon* C. H. Müll., with four species bearing pubescent green or greenish white to yellowish and purple tinged fruits, frequently with a dark green, lavender, or purple stripe; thick glabrous seeds or pilose only at the apex; bracteate inflorescences; and leaves usually with pseudostipules. Müller (1940) also described *L. glandulosum* C. H. Müll. and classified the highly polymorphic *L. peruvianum* into two varieties: var. *dentatum* Dunal and var. *humifusum* C. H. Müll.

Luckwill (1943; Fig. 1) adopted the two subgenera recognized by Müller (1940) and proposed a phylogeny of *Lycopersicon*. He hypothesized that the two subgenera might have evolved from an ancestral simple form characterized by imparipinnate leaves with 5 to 7 entire leaflets, few interjected leaflets, and probably no secondary leaflets, unbranched inflorescences, and

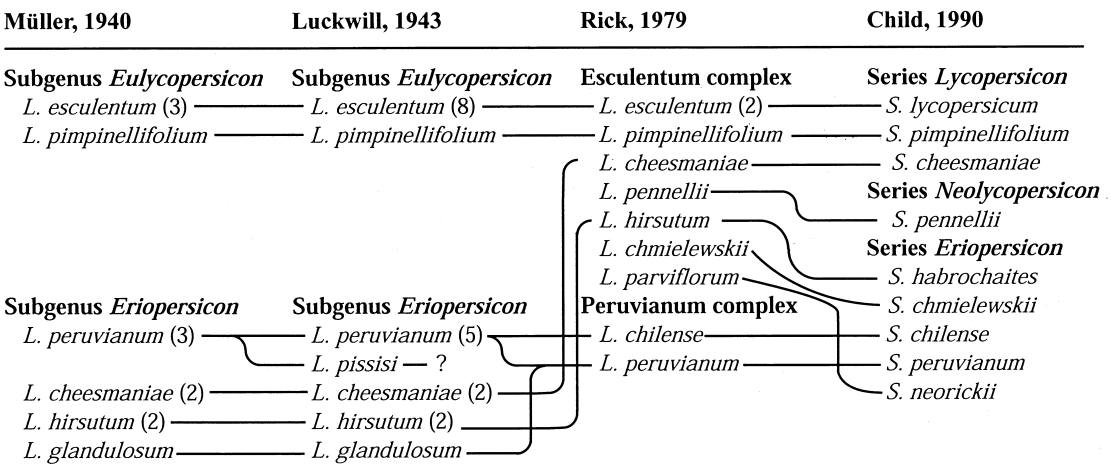

Figure 1. Comparison of classifications of *Solanum* sect. *Lycopersicon*. The numbers in parentheses represent numbers of infraspecific ranks (subspecies, varieties, and forms). The lines connect synonymous taxa.

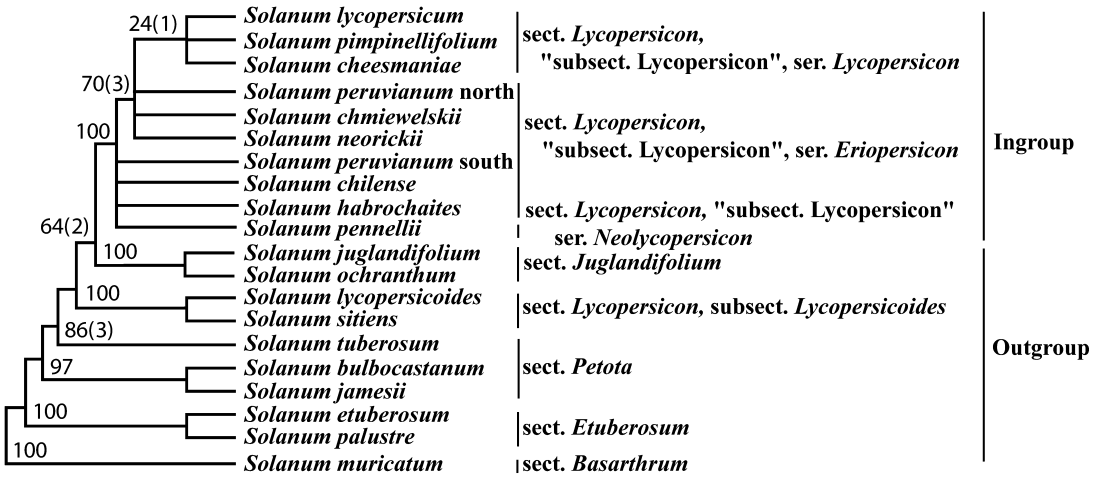

Figure 2. Abstracted cladistic results of the 65 accessions of the 9 tomato species and 14 outgroup taxa examined in the phylogenetic analysis of the GBSSI gene sequences by Peralta and Spooner (2001). Nomenclature of *Solanum* subg. *Potatoe* according to Child (1990). We here informally label wild tomatoes in the clade containing all members of "subsect. Lycopersicon" (*S. lycopersicum* to *S. pennellii*) to provide a coordinate name for their formally described outgroups in section *Juglandifolium* (Rydb.) Child and subsection *Lycopersicoides* Child. Our use of these ranks may change in our monograph of tomatoes and their relatives (Peralta et al., in prep.). Numbers indicate bootstrap values, and decay values are indicated between parentheses.

undeveloped pseudostipules. Two different lineages diverged from the ancestral forms, one characterized by fruits with carotenoid pigments and the other by green fruits with anthocyanin pigments. Luckwill (1943) agreed with Müller (1940) in the circumscription of subgenus *Eulycopersicon*, but within *Eriopersicon* he considered *L. pissisi* a distinct species, with *L. peruvianum* var. *humifusum* C. H. Müll. as its basionym (Fig. 1). He also proposed different infraspecific categories in *L. esculentum*, *L. cheesmaniae*, and *L. peruvianum*. *Lycopersicon cheesmaniae*, with yellow to orange fruits, bractless inflorescences, and leaves without pseudostipules, apparently was misclassified in the subgenus *Eriopersicon* by Müller (1940) and Luckwill (1943).

D'Arcy (1972) treated tomatoes in the genus *Lycopersicon* but did not address subgeneric relationships. More recently, Child (1990, Fig. 1) placed tomatoes in *Solanum* subg. *Potatoe* (G. Don) D'Arcy, sect. *Lycopersicon* (Mill.) Wettst., subsect. *Lycopersicon*, mainly characterized by anthers with sterile appendages and laterally connivent forming a tube, and classified them in three series: *Lycopersicon*, *Eriopersicon* (C. H. Müll.) Child, and *Neolycopersicon* (Correll) Child (Fig. 1). The first two series correspond to the subgenera *Eulycopersicon* and *Eriopersicon* used by Müller (1940) and Luckwill (1943). Series *Lycopersicon* includes herbs with few or no pseudostipules, ebracteate inflorescences, and fruits with carotenoid pigments, and *Eriopersicon* includes herbs to subshrubs with pseudostipules, bracteate inflorescences, and fruits with no carotenoid pigments (fruits pure green or greenish white, dark green to purple striped). Series *Neolycopersicon* only includes *S. pennellii* (Correll, 1958), a species with curved, markedly unequal length anthers loosely coherent and without sterile appendages. Child (1990) also proposed *Solanum* sect. *Lycopersicon* (Mill.) Wettst., subsection *Lycopersicoides* Child, and section *Juglandifolium* (Rydb.) Child as the closest relatives of subsection *Lycopersicon*.

Most recently, a new orange-fruited species of tomato was recognized, *S. galapagense* S. Darwin & Peralta (Darwin et al., 2003). Within tomatoes it shares its orange fruit color only with *S. cheesmaniae*, but is clearly distinguished from it by a number of leaf and flower traits. This species, formerly recognized as *S. cheesmaniae* f. *minor*, is clearly related to the other species with carotenoid pigments and is included in the present study.

Mating systems have played an important role in the evolution of wild tomato species, varying from allogamous self-incompatible, to facultative allogamous and self-compatible, to autogamous and self-compatible (Rick, 1963, 1979, 1982b, 1986b; Table 1). The self-incompatibility system in tomatoes is gametophytic and controlled by a single, multiallelic S locus (Tanksley & Loaiza-Figueroa, 1985).

The self-incompatibility system has shown strong relationships between the extent of outcrossing and allelic polymorphisms, floral display, and degree of stigma exsertion in wild tomatoes. Rick (1982b) investigated the genetic bases of self-compatibility, self-incompatibility, and flower characters, using interspecific hybrids between the self-compatible (SC) *S. pimpinellifolium*, used as recurrent parent, and the two self-incompatible (SI) species, *S. habrochaites* and *S. pennellii*. He postulated that three independent genetic phases, most probably regulated by different unlinked genes or gene complexes, are essential for successful functioning of the self-incompatibility system. These genes are operating on (1) prevention of self-fertilization, (2) changes in the flower organs to ensure cross-pollination, and (3) development of secondary flower characters to attract pollinators. He concluded that the evolution of a mating system in wild tomatoes has occurred from self-incompatibility, as ancestral condition, to self-compatibility, and probably never reversed to self-incompatibility. Changes from self-incompatibility to self-compatibility are expected to arise frequently and independ-

ently (Rick, 1982b). This trend has been found in *S. habrochaites* and *S. pennellii*, both with self-incompatible and self-compatible populations. The self-incompatible populations occupy the center of their species geographic distributions, and have higher genetic variation, larger flower parts, and exserted stigmas. Self-compatible populations occur toward the northern and southern edges of *S. habrochaites* and *S. pennellii* distribution, have less genetic variation, smaller flower parts, and little or no stigma exsertion (Rick et al., 1979; Rick & Tanksley, 1981). The change from self-incompatibility to self-compatibility has been reported in only one population of *S. peruvianum* (Rick, 1986b).

In the self-compatible species, the extent of outcrossing and genetic variation is also related to floral display and degree of stigma exsertion. Marginal populations of *Solanum pimpinellifolium* are highly autogamous with little or no genetic variation, small flower parts, and little or no stigma exsertion, while the central facultative allogamous populations have high genetic variation, larger corollas, and marked stigma exsertion (Rick et al., 1977). A comparison of different *S. pimpinellifolium* genotypes in experimental plots in Peru showed that different outcrossing rates could be largely attributed to differences in floral characters, especially the level of stigma exsertion, rather than to differences in numbers and types of pollinators (Rick et al., 1978).

Two closely related self-compatible species, *S. chmielewskii* and *S. neorickii*, illustrate another example of changes in flower characters associated with outcrossing and genetic variation. *Solanum neorickii* is exclusively autogamous with low intra-population genetic variation and small flowers with stigmas included in the anther tube. In contrast, the facultative allogamous *S. chmielewskii* exhibited higher levels of heterozygosity, larger flower parts, and exserted stigmas. Rick et al. (1976) postulated that *S. neorickii* evolved from *S. chmielewskii*. All populations of *S. chilense* are self-incompatible. The related species *S. lycopersicoides*, *S. sitiens*, *S. ochranthum*, and *S. juglandifolium* are exclusively self-incompatible.

In contrast to the morphological species concepts of Müller (1940) and Luckwill (1943), Rick (1960, 1963, 1979, 1986b) recognized species and proposed a supraspecific classification based primarily on the biological criteria of mating systems, cytology, genetic variation, and crossing relationships. He recognized nine species of *Lycopersicon* (Fig. 1; Rick, 1979), including two new species, *L. parviflorum* and *L. chmielewskii* (Rick et al., 1976), and *L. pennellii* (Rick, 1979). Furthermore, *L. chilense*, a species described by Dunal (1852), was revalidated by Rick and Lamm (1955), and *L. glandulosum* was included as a synonym of *L. peruvianum*.

In order to determine the crossing relationships necessary for his tomato breeding program, Rick (1963, 1979, 1986b) hybridized numerous accessions of all tomato species in all possible combinations and found two major crossing complexes. The Esculentum complex included seven species, mainly self-compatible and intercrossable (Fig. 1; Table 1). *Lycopersicon hirsutum* and *L. pennellii* typically are self-incompatible, but with some self-compatible populations. Three species of the Esculentum complex have mostly glabrous, red- to orange- to yellow-colored fruits (*L. cheesmaniae*, *L. esculentum*, *L. pimpinellifolium*), while the others have pubescent, green fruits (*L. chmielewskii*, *L. hirsutum*, *L. parviflorum*, *L. pennellii*). Hybrids were obtained in all combinations, in spite of the presence of some unilateral incompatibility. The Peruvianum complex, on the other hand, included the self-incompatible species *L. chilense* and *L. peruvianum* (Fig. 1; Table 1), both with pubescent green fruits. The barrier between these two complexes can be broken only by embryo rescue (Rick, 1979). The chromosomes of the F_1 hybrids of successful inter-complex crosses generally showed complete pairing at pachytene, and normal chiasmata formation and chromosome segregation at anaphase during meiosis. Rick (1979) concluded that speciation in wild tomatoes took place mainly via gene substitution and, to a minor extent, by chromosomal differentiation. He also pointed out that natural hybridization is very

TABLE 1.

Accessions of wild tomatoes and outgroups, examined for morphological (this paper) and GBBSI gene sequence variation (Peralta & Spooner, 2001). Vouchers are deposited at BM, MERL, PTIS, and WIS. We used the *Solanum* equivalents of the *Lycopersicon* species as recognized by Rick et al. (1990), with the addition of the varieties and forms of *S. habrochaites*, *S. pennellii*, and *S. peruvianum* as listed in the database of germplasm at the C. M. Rick Tomato Genetics Resource Center (<http://tgrc.ucdavis.edu>).

Taxon[a]	Map Locality[b]	Accession Number[c]	Collector[d]	Breeding System[e]	Locality[f]
Ingroup, *Solanum* sect. *Lycopersicon*					
chs	G	LA166	Unknown	Aut-SC	Ecuador. Galápagos Islands: Santa Cruz, Barranco, N of Punta Ayora, 1 km N of Punta Ayora, 50 m
chs	G	LA1450	Unknown	Aut-SC	Ecuador. Galápagos Islands: Isabela, Bahia San Pedro, 1/2 km from coast, 15 m
gal	G	LA317	Unknown	Aut-SC	Ecuador. Galápagos Islands: Bartolome, 15 m
chl	29	LA1930	*Rick et al. 3172*	Allo-SI	Peru. Arequipa: Quebrada Calapampa, Río Acarí, 500 m, 15°39'S, 74°39'W
chl	36	LA1963	*Rick et al. 3204*	Allo-SI	Peru. Tacna: Río Caplina, 13 km W of Tacna, 3 km from Panamerican Hwy., toward Boca del Río, 200 m, 18°14'S, 70°33'W
chl	38	LA2884	*Rick et al. 7609*	Allo-SI	Chile. Antofagasta: Ayaviri, 22°02'S, 68°07'W
chm	25	LA1306 (PI 379029)	*Rick et al. 390*	Fac-SC	Peru. Ayacucho: Tambo, 12°56'S, 74°01'W, 1 km E of Tambo, 3100 m
chm	27	LA1327	*Rick et al. 411*	Fac-SC	Peru. Apurímac: Soracata, 1980 m
chm	26	LA2663	*Rick et al. 6101*	Fac-SC	Peru. Cusco: Tujtohaiya (Upper Apurímac), 2500 m
lypc	5	LA1226 (PI 379045)	*Rick et al. 309*	Aut-SC	Ecuador. Morona-Santiago: 3 km S of Sucua, 1000 m
lypc	21	LA1673	*Rick et al. 1985*	Aut-SC	Peru. Lima: Ñaña, 11°59'S, 76°50'W, along W wall of the Estancia
hab	11	LA1353 (PI 365934)	*Rick et al. 437*	Allo-SI	Peru. Cajamarca: Contumazá, 2650 m, 7°22'S, 78°49'W
hab	24	LA1928	*Rick et al. 3170*	Fac-SC	Peru. Ica: Ocaña, Río Ingenio, 76 km from Panamerican Hwy., 2660 m, 14°39'S, 75°15'W
habg	3	LA1223 (PI 365903)	*Rick et al. 301*	Fac-SC	Ecuador. Chimborazo: Alausí, 2°12'S, 78°50'W, 2200 m
neo	7	LA2200	*Rick et al. 5045*	Aut-SC	Peru. Amazonas: Choipiaco, Río Suche, 6°13'S, 77°51'W, 16 km E of Chachapoyas, 1900 m
neo	17	LA247	*Ochoa 1017*	Aut-SC	Peru. Huánuco: Chavinillo, 9°47'S, 76°35'W

neo	20	LA1326 (PI 379033)	*Rick et al. 410*	Aut-SC	Peru. Apurímac: Río Pachachaca, 1980 m, 11°39′S, 76°01′W
pen	19	LA1376 (PI 379017)	*Rick et al. 460*	Allo-SI	Peru. Lima: Sayán, 1000 m, Agua Perdida (Río Ingenio), 11°06′S, 77°39′W
pen	31	LA716 (PI 246502)	Unknown	Fac-SC	Peru. Arequipa: Atico, near Atico from Chala along Panamerican Hwy., 16°14′S, 73°39′W
penp	28	LA1926	*Rick et al. 3168*	Allo-SI	Peru. Ica: 25 km from Panamerican Hwy., 1200 m, 14°38′S, 75°04′W
per	6	LA2185	*Rick et al. 5030*	Allo-SI	Peru. Amazonas: Pongo de Rentema, in the gap of the Marañón, E of Corral Quemado, 400 m, 5°26′S, 79°39′W
per	9	LA2172	*Rick et al. 5017*	Allo-SI; possibly SC or segregating SI/SC	Peru. Cajamarca: Cuyca, 161 km from Olmos junction with the Panamerican Hwy., 11°31′S, 75°07′W
per	9	LA2163	*Rick et al. 5008*	Allo-SI	Peru. Cajamarca: between Cochabamba and Yamaluc, 2–5 km N of Cochabamba, 1800–1900 m
per	9	LA2157	*Rick et al. 5002*	Fac-SC	Peru. Cajamarca: Tunel Chotano, 23 km N of Chota, 1600 m
per	11	LA392	*Rick et al. 141*	Allo-SI	Peru. Cajamarca: Llallan, 78 km from Car. Panamerican Hwy., 12 km W of Chile, 900 m
perh	11	LA2152	*Rick et al. 4997*	Allo-SI	Peru. Cajamarca: Río Jequetepeque, 140 km from Panamerican Hwy., 2 km W of San Juan, 2150 m, 7°17′S, 78°29′W
perh	11	LA2548	PE-29	Allo-SI	Peru. Cajamarca: La Muyuna, Río Jequetepeque, 870 m
per	10	LA1396	*Rick et al. 480*	Allo-SI	Peru. Amazonas: Balsas, 4°25′S, 80°17′W
per	13	LA2553	PE-31	Allo-SI	Peru. Cajamarca: Balconcillo-San Marcos, 2650 m, 7°20′S, 78°11′W
per	12	LA441	*Rick et al. 128*	Allo-SI	Peru. La Libertad: Cerro Campana, W of Panamerican Hwy. N of Trujillo, 350 m, 5°05′S, 79°17′W
per	12	LA1984	*Rick et al. 3225*	Allo-SI	Peru. La Libertad: Otuzco (Río Moche), 2 km on side road E of Otuzco, 2800 m, 7°54′S, 78°35′W
per	13	LA2328	*Rick et al. 5172*	Allo-SI	Peru. La Libertad: Aricapampa, 2300–2500 m, 7°48′S, 77°43′W
per	14	LA1982	*Rick et al. 3223*	Allo-SI	Peru. Ancash: Huallanca, Río Santa, near the hydroelectric plant, about 4 km beyond LA1981, 1400 m, 8°49′S, 77°52′W
per	15	LA1626	Unknown	Allo-SI	Peru. Ancash: mouth of Río Rupac, 1700 m
per	16	LA1360 (PI 365952)	*Rick et al. 444*	Allo-SI	Peru. Ancash: Pariacoto, 1490 m, 9°31′S, 77°53′W
per	18	LA111	*Rick et al. 41*	Allo-SI	Peru. Lima: Supe, along Panamerican Hwy. at km 158, 50 m, 10°48′S, 77°44′W
per	18	LA110	*Rick et al. 39*	Allo-SI	Peru. Ancash: Cajacay, 5 km W of Cajacay, 2500 m, 10°10′S, 77°26′W

TABLE 1 CONTINUED.

Taxon[a]	Map Locality[b]	Accession Number[c]	Collector[d]	Breeding System[e]	Locality[f]
per	18	LA1365 (PI 365953)	*Rick et al. 449*	Allo-SI	Peru. Ancash: Caranquilloc, 2450 m
per	19	LA1379 (PI 379018)	*Rick et al. 463*	Allo-SI	Peru. Lima: Caujul, 1500 m, 10°48′S, 77°02′W
per	21	LA1274 (PI 365940)	*Rick et al. 356*	Allo-SI	Peru. Lima: Pacaibamba, 1440 m
per	21	LA370	*Rick et al. 115*	Allo-SI	Peru. Lima: Huampani Quebrada, 200 m
perg	21	LA364	*Rick et al. 109*	Allo-SI	Peru. Lima: 9 km W of Canta, 2100 m, 12°35′S, 69°09′W
perg	21	LA1283 (PI 365942)	*Rick et al. 365*	Allo-SI	Peru. Lima: Santa Cruz de Laya, 2000 m
per	21	LA103	*Rick et al. 28*	Allo-SI	Peru. Lima: Cajamarquilla, on both sides of dry wash, 100 m, 6°25′S, 79°14′W
per	22	LA107	*Rick et al. 33*	Allo-SI	Peru. Lima: Hacienda San Isidro, 5°33′S, 80°49′W
per	23	LA1339 (PI 365949)	*Rick et al. 423*	Allo-SI	Peru. Lima: Capillucas, 1700 m, 13°39′S, 12°45′W
per	23	LA444	*Rick et al. 198*	Allo-SI	Peru. Ica: Chincha, southern outskirts, 100 m, 17°20′S, 71°00′W
per	24	LA1305 (PI 379015)	*Rick et al. 389*	Allo-SI	Peru. Huancavelica: 20 km E of Ticrapo, 2960 m
per	24	LA1910	*Rick et al. 3152*	Allo-SI	Peru. Huancavelica: Ticrapo, Tambillo (Río Ica), 74 km from Ica, 2300 m
per	28	LA1913	*Rick et al. 3155*	Allo-SI	Peru. Ica: Tinguiayog (Río Santa Cruz), 15 km from Panamerican Hwy., 900 m
per	28	LA1331 (PI 365946)	*Rick et al. 415*	Allo-SI	Peru. Ica: Nazca, 1500 m, 14°50′S, 74°57′W
per	29	LA1937	*Rick et al. 3179*	Allo-SI	Peru. Arequipa: Quebrada Torrecillas, (Río Chaparra), 21 km from Chaparra, 64 km from Panamerican Hwy., 2500 m, 15°55′S, 74°07′W
per	30	LA448	*Rick et al. 202*	Allo-SI	Peru. Arequipa: Chala, 15°52′S, 74°16′W
per	30	LA1945	*Rick et al. 3186*	Allo-SI	Peru. Arequipa: Caravelí, 71 km from Panamerican Hwy., 8 km before reaching Caravelí, 1940 m, 15°46′S, 73°22′W
per	32	LA1474	Unknown	Allo-SI	Peru. Arequipa: Lomas de Camaná, 1300 m, 16°22′S, 73°01′W
per	33	LA1973	Unknown	Allo-SI	Peru. Arequipa: Yura, 16°12′S, 71°42′W
per	35	LA3156	Unknown	Allo-SI	Peru. Moquegua: Omate Agricultural Valley
per	34	LA1954	*Rick et al. 3195*	Allo-SI	Peru. Arequipa: 2–4 km W of Mollendo, 50 m, 17°02′S, 72°01′W

per	34	LA454	*Rick et al. 208*	Allo-SI	Peru. Arequipa: 8 km S of Río Tambo crossing, 1200 m
per	36	LA2964	Unknown	Allo-SI	Peru. Tacna: Quebrada de Los Burros
per	37	LA2744	*Rick et al. 7502*	Allo-SI	Chile. Tarapaca: Sobraya, (Azapa), 31 km from Arica, 400 m
pim	2	LA1237 (PI 365910)	*Rick et al. 318*	Aut-SC	Ecuador. Esmeraldas: Atacames, 5 m
pim	8	LA1581	*Rick et al. 1861*	Fac-SC	Peru. Lambayeque: Punto Cuatro, 1 km W of Panamerican Hwy.
pim	23	LA1606	*Rick et al. 1886*	Aut-SC	Peru. Ica: Tambo de Mora, Huaucas, 13°28'S, 76°12'W
Outgroup, *Solanum* sect. *Lycopersicum* subsect. *Lycopersicoides* Child					
lyc	36	LA2386	*Ochoa 14248*	Allo-SI	Peru. Tacna: Chupapalca, 3180 m
sit	39	LA2876	*Rick et al. 7601*	Allo-SI	Chile. Antofagasta: Chuquicamata

[a]Species abbreviations [and *Lycopersicon* synonyms] are:

chl = *S. chilense* (Dunal) Reiche [*Lycopersicon chilense* Dunal];

chm = *S. chmielewskii* (C. M. Rick, Kesicki, Fobes & M. Holle) D. M. Spooner, G. J. Anderson & R. K. Jansen [*L. chmielewskii* C. M. Rick, Kesicki, Fobes & M. Holle];

chs = *S. cheesmaniae* (L. Riley) Fosberg [*L. cheesmaniae* L. Riley];

gal = *S. galapagense* S. Darwin & Peralta [*L. cheesmaniae* f. *minor* (Hook. f.) C. H. Müll.] ;

hab = *S. habrochaites* S. Knapp & D. M. Spooner [*L. hirsutum* Dunal];

habg = *S. habrochaites* f. *glabratum* C. H. Müll.;

lyc = *S. lycopersicoides* Dunal [*L. lycopersicoides* (Dunal) A. Child ex J. M. H. Shaw];

lyp = *S. lycopersicum* L.;

lypc = *S. lycopersicum* L. var. *cerasiforme* (Dunal) D. M. Spooner, G. J. Anderson & R. K. Jansen [*L. esculentum* Mill. var. *cerasiforme* Dunal];

neo = *S. neorickii* D. M. Spooner, G. J. Anderson & R. K. Jansen [*L. parviflorum* C. M. Rick, Kesicki, Fobes & M. Holle];

pen = *S. pennellii* Correll [*L. pennellii* (Correll) D'Arcy];

penp = *S. pennellii* var. *puberulum* Correll;

per = *S. peruvianum* L. [*L. peruvianum* (L.) Miller];

perg = *L. peruvianum* f. *glandulosum* C. H. Müll.;

perh = *L. peruvianum* var. *humifusum* C. H. Müll.;

pim = *S. pimpinellifolium* L. [*L. pimpinellifolium* (L.) Miller];

sit = *S. sitiens* I. M. Johnst. [*L. sitiens* (I. M. Johnst.) J. M. H. Shaw];

[b]Map numbers correspond to Figure 1; G refers to unmapped accessions from the Galápagos Islands located about 1000 km west of Ecuador.

[c]LA numbers are from the C. M. Rick Tomato Genetics Resource Center; PI numbers are the same United States Plant Introduction Number duplicate accessions held at the United States Department of Agriculture genebank in Geneva, New York (Geneva Plant Genetic Resources Unit, NE-9).

[d]The collector abbreviation in the database of the C. M. Rick Tomato Genetics Resource Center is SAL, an abbreviation used by Rick for his collections alone or with others to indicate "South American *Lycopersicon*."

[e]Allo = allogamous, Aut = autogamous, SC = self-compatible, SI = self-incompatible.

[f]Longitude and latitude data added by the authors.

unlikely, and only a few cases have been reported of spontaneous introgression between the two closely related *L. esculentum* and *L. pimpinellifolium* (Rick, 1986b).

In addition, Rick (1963, 1986b) recognized 40 informally designated races or ecotypes in the polymorphic *S. peruvianum*. A few of these are widespread coastal races, but the majority are locally distributed montane races. He proposed that strict gametophytic self-incompatibility and geographic isolation drove differentiation among the *S. peruvianum* races (Rick, 1986b). Hybridization showed an almost complete reproductive barrier between variety *humifusum*, a race growing at high altitude (2500 m) in the Jequetepeque River in northeast Peru, and most of the other *S. peruvianum* populations. Nevertheless, the reproductive isolation was not complete, due to the existence of some bridge races (races theoretically able to maintain gene flow between northern and southern races) located in central Peru (Rick, 1963, 1986b).

Studies of additional collections and crosses of northern populations of *Lycopersicon peruvianum* allowed Rick (1986b) to identify four groups of races that were isolated by reproductive barriers. Three groups of races occur in northern Peru: the Chamaya-Cuvita group of races, the Marañón group of races, and Chotano-*humifusum* group of races. The fourth group of races comprised all the remaining races of *L. peruvianum* from central and southern Peru. Rick (1986b) confirmed the crossing barriers between the northern and southern races, and found that some of the northern races crossed to a limited degree with *L. chilense*, *L. hirsutum*, and *L. esculentum*. Based on these findings, Rick (1986b) hypothesized that the Río Marañón races of *L. peruvianum* were ancestral to all other wild tomatoes.

The Endosperm Balance Number (EBN) crossability phenomenon was analyzed for Rick's two wild tomato complexes by Ehlenfeldt and Hanneman (1992). The EBN hypothesis (Johnston et al., 1980; Ortiz & Ehlenfeldt, 1992; Hanneman, 1994) postulates that in the absence of stylar barriers, the success or failure of a cross is determined primarily by a 2:1 maternal to paternal balance in the endosperm, independent of ploidy. The EBN is determined using standard crossing species with known EBNs. The EBN data supported the hypothesis of two intra-fertile groups as proposed by Rick (1979). The Esculentum complex showed uniformity of EBN values, which can be compared to the 2x(1EBN) species in potato. On the other hand, the Peruvianum complex showed variable values for EBN, but most comparable to 2x(2EBN) potato species (Ehlenfeldt & Hanneman, 1992). These authors hypothesized that the Esculentum and Peruvianum complexes are separated by a system analogous to the 2x(1EBN) *S. commersonii* and 2x(2EBN) *S. chacoense* crossability groups. This isolating mechanism may restrict or suppress gene flow among sympatric populations, and may play a role in the reproductive isolation in tomatoes like in the *L. peruvianum* var. *humifusum* races from northern Peru (Ehlenfeldt & Hanneman, 1992).

Molecular data from chloroplast DNA restriction site and sequence data (Spooner et al., 1993; Bohs & Olmstead, 1997, 1999; Olmstead & Palmer, 1997; Olmstead et al., 1999) firmly place tomatoes and potatoes as sister taxa. The systematic placement of tomatoes under *Solanum*, as adopted here, is beginning to gain acceptance in the taxonomic literature (Bohs & Olmstead, 1997, 1999; Olmstead & Palmer, 1997), but it is still controversial and highlights competing goals and hypotheses of classification (Peralta & Spooner, 2000).

Peralta and Spooner (2001) examined interspecific relationships of all 10 wild tomato species, with a concentration on the highly polymorphic and widespread species *Solanum peruvianum*, with DNA sequence data of the single-copy nuclear encoded GBSSI gene. Their outgroup results were concordant with the cpDNA restriction fragment length polymorphism (RFLP) phylogeny of Spooner et al. (1993), supporting *S. juglandifolium*, *S. lycopersicoides*, and *S. sitiens*

as outgroups. The ingroup of wild tomatoes showed a basal polytomy and a terminal clade. The basal polytomy was composed of the self-incompatible green-fruited species *S. chilense*, the central to southern Peruvian populations of *S. peruvianum*, *S. habrochaites*, and *S. pennellii*. The terminal clade contained the northern Peruvian populations of *S. peruvianum* (also self-incompatible, green fruits), *S. chmielewskii*, and *S. neorickii* (self-compatible, green fruits), and the self-compatible and red- to orange- to yellow-fruited species *S. cheesmaniae, S. galapagense, S. lycopersicum*, and *S. pimpinellifolium* (Fig. 2). The results supported allogamy, self-incompatibility, and green fruits as plesiomorphic in tomatoes.

We wished to examine morphology as an independent and taxonomically accessible data set, using the same accessions in the GBSSI study (Peralta & Spooner, 2001). All prior classifications used morphology for intuitive taxonomic judgments, and this is the first explicit use of morphology for phenetic and cladistic analyses in tomatoes. The goals of this study are: (1) to examine interrelationships among all 10 wild tomato species with phenetic and cladistic analyses of morphological data, and (2) to compare our results with the morphological (Müller, 1940; Luckwill, 1943) and biological (Rick, 1979; Rick et al., 1990) classifications; mitochondrial DNA restriction site results (McClean & Hanson, 1986); nuclear DNA RFLP results (Miller & Tanksley, 1990), cpDNA restriction site results of Palmer and Zamir (1982) and Spooner et al. (1993), and GBSSI DNA sequence results (Peralta & Spooner, 2001). Our results show diagnostic characters useful for species differentiation for a monographic study of *Solanum* sect. *Lycopersicon*, currently in progress.

Materials and Methods

SPECIES

For phenetic analyses, we analyzed a total of 66 accessions of all 10 species of tomatoes (Table 1), including two species of *Solanum* subsect. *Lycopersicoides* supported as a close outgroup of tomato (Rick, 1979, 1988; Child, 1990; Spooner et al., 1993). All the accessions, representing much of the ingroup variation, were obtained from the C. M. Rick Tomato Genetics Resource Center, Department of Vegetable Crops, University of California, Davis. The late Charles Rick, former curator of this gene bank, kindly provided advice on the choice of accessions, based on geographic distribution, morphology, genetic diversity, and breeding behavior (Fig. 3; Table 1). He also advised us on critical morphological characters to differentiate taxa (Table 2). The accessions represent nearly the same as those used in the GBSSI gene phylogenetic analysis of Peralta and Spooner (2001).

Three accessions per species were analyzed for *Solanum chmielewskii*, *S. chilense*, *S. habrochaites*, *S. neorickii*, *S. pennellii*, *S. pimpinellifolium*, two accessions for *S. lycopersicum* var. *cerasiforme* and *S. cheesmaniae*, one accession of *S. galapagense*, and 41 accessions for *S. peruvianum*. One accession of *S. lycopersicoides* and one accession of *S. sitiens* were included as closely related non-tomato comparison species. For cladistic analyses, we determined taxon-specific characters (Tables 3, 4) based on results from the character state variation and phenetic analyses.

The plants were grown in the fall, because some wild tomato species need short photoperiods to flower. Seeds were planted in greenhouses of the University of Wisconsin, Madison, in mid August 1999. Two weeks later, seedlings were transferred to 30 cm diameter plastic pots with sterilized soil. Three plants per accession were randomly distributed in two replicates, and a total of six individuals were evaluated per accession. Stem characters and leaves were measured from the ninth node, when plants developed more than 12 nodes and were usually between 60 and 80 cm high. Most of the plants began to flower by the end of September or the first week of October. Flowers were collected from mature inflorescences when the plants were in full bloom, during the months of October, November, and December. In order to

Table 2.

Morphological characters used in the phenetic analysis of wild tomatoes and outgroups. All measurements are in millimeters, except as noted below. Characters marked with an asterisk (*) were not significantly different ($P = 0.05$) between at least two species under the Tukey-Kramer HSD test.

STEM CHARACTERS

1. Stem diameter at base. 2. Internode length between the 9th and 10th node (cm). 3. Number of branches in the first 20 cm at flowering time. 4. Number of nodes before branch bifurcation. 5. Plant height at branch bifurcation (cm). 6. Pseudostipules: absent (0); present (1). 7. Number of leaves per sympodium.

LEAF CHARACTERS

8. Leaf length (cm). 9. Ratio: leaf length/leaf width. 10. Ratio: leaf length/length of leaf from widest point to leaf apex. 11. Leaf petiole length (cm). 12. Terminal leaflet lamina length (cm)*. 13. Ratio: length of terminal leaflet lamina/width of lamina. 14. Ratio: length of terminal leaflet lamina/length of terminal leaflet lamina from its widest point to apex. 15. Terminal leaflet petiolule length (cm)*. 16. Width of terminal leaflet lamina from a point 5 mm below the apex (cm). 17. Length of primary dorsal leaflet lamina (cm). 18. Ratio: length of primary dorsal leaflet lamina/width of lamina. 19. Ratio: length of primary dorsal leaflet lamina/length of primary dorsal leaflet lamina from its widest point to apex. 20. Length of primary dorsal leaflet petiolule. 21. Width of primary dorsal lateral leaflet lamina from a point 5 mm below the apex. 22. Number of lateral leaflets. 23. Number of interjected leaflets. 24. Number of secondary leaflets. 25. Number of tertiary leaflets.

INFLORESCENCE CHARACTERS

26. Inflorescence bracts: absent (0); present (1). 27. Number of branches per mature inflorescence. 28. Number of flowers per branch*. 29. Length of the mature inflorescence axis (cm). 30. Ratio: number of flowers/length of inflorescence axis. 31. Peduncle length (cm). 32. Ratio: total inflorescence length/peduncle length.

FLOWER CHARACTERS

33. Pedicel length (cm). 34. Ratio: pedicel length from base of the pedicel to articulation/pedicel length. 35. Sepal length. 36. Ratio: sepal length/width. 37. Length from center of corolla to apex of corolla lobe (cm). 38. Ratio: length from center of corolla to apex of corolla lobe/length from center of corolla to base of corolla lobe. 39. Width of corolla lobe at base of corolla lobe junction (cm)*. 40. Ratio: length from a line drawn across the widest point of corolla lobe to lobe apex/width of corolla lobe at base of corolla lobes junction. 41. Anther length. 42. Anther appendage length. 43. Anther filament length. 44. Anther appendage curvature: straight (1), slightly curved to 40˚ (2), curved more than 45˚ (3). 45. Anther attachment: free (0), attached (1). 46. Anther dehiscence: pores (0), longitudinal split (1). 47. Anther color: white (0), yellow (1). 48. Style length exsertion from apex of anthers to stigma apex. 49. Stigma width. 50. Ovary length. 51. Style length (cm).

FRUIT CHARACTERS

52. Diameter at its widest dimension. 53. Ratio: diameter at widest dimension/diameter at narrowest dimension*. 54. Ratio: diameter at widest dimension/fruit length. 55. Fruit color: green (1), pale green (2), yellow green (3), orange (4), red (5). 56. Fruit stripe: absent (0); present (1). 57. Fruit pubescence: glabrous to glabrescent (1), pubescent to pilose (2), hirsute (3).

PLANT TRICHOMES

See Luckwill (1943) for illustrations of trichome types, and materials and methods for the method of scoring.

58. Type I. 59. Type II. 60. Type III. 61. Type IV. 62. Type V. 63. Type VI. 64. Type VII*. 65. Type VIII*. 66. Trichome density.

obtain fruits from the self-incompatible (SI) accessions, plants were pollinated manually using pollen of different individuals belonging to the same accessions. Self-compatible (SC) accessions produced fruits naturally. Fruits were harvested approximately two months after pollination. Vouchers are deposited at DAV, MERL, PTIS, and WIS.

DATA MEASUREMENT

For phenetic analyses, we assessed a total of 66 characters (50 quantitative and 16 qualitative) for 6 individuals of 66 accessions (over 26,000 data points). The quantitative characters included 14 ratios that assessed shapes of different plant organs (Table 2). Trichomes were studied from young stems by making thin slice cuts 1 or 2 cm below the apex with a razor blade. Trichome types were identified according to the classification of Luckwill (1943) using a transmission light microscope (100× magnification). An arbitrary scale based on percentages: 1%–20% = 1, 21%–40% = 2, 41%–60% = 3, 61%–80% = 4, 81%–100% = 5, was used to score the frequency of different trichomes (characters 58 to 65). The diameter and height of the thin stem slice were measured under a microscope, and the number of trichomes around the slice circumference was counted. Trichome density percentage (D = n/S 100; character 66) was estimated as the total number of trichomes (n) divided by the cylinder square ($S = \pi \times \text{diameter}/2 \times \text{height}$), represented by the thin stem slide.

For cladistic analyses, we chose characters that were shown by our analysis of character state variation (below) to have non-overlapping or nearly non-overlapping states (Table 3).

DATA ANALYSIS:
CHARACTER STATE VARIATION AND PHENETICS

The mean value of the six plants per accession was used as the Operational Taxonomic Unit (OTU). The mean, range, and standard deviation were estimated for each character. Significant character state differences ($P = 0.05$) between any two pairs of species were evaluated using the Tukey-Kramer test with JMP statistical software (SAS, 1995).

Cluster analyses were produced by NTSYS-pc^R version 1.70 (Rohlf, 1992) only on the subset of 66 characters found to be statistically significant among groups by one-way ANOVA. Averages for each character were standardized (STAND), and similarity matrices, using average taxonomic distance (DIST), Manhattan distance (MANHAT), Euclidean distance (EUCLID), and product-moment correlation (CORR) were generated. Clustering was performed using the unweighted pair-group method (UPGMA) in SAHN. Cophenetic correlation coefficients (COPH and MXCOMP) were used to measure distortion between the similarity matrices and the resultant three phenograms (Rohlf & Sokal, 1981; Sokal, 1986). Stepwise discriminant analysis (SDA) was performed on all 66 characters by SAS Version 7 (SAS, 1998), using the mean values.

Four entities in *Solanum peruvianum* (the northern and southern populations, f. *glandulosum,* and var. *humifusum*), *S. cheesmaniae,* and *S. galapagense* (= *S. cheesmaniae* f. *minor*), were recognized as OTUs to estimate the mean, range, and standard deviation. In addition to these taxa, two forms of *S. habrochaites* (f. *typicum* and f. *glabratum*) and two varieties of *S. pennellii* (var. *typicum* and var. *puberulum*) were considered in the cluster analysis and in the SDA. The forms and varieties of the latter two species are mainly differentiated by their pubescence.

DATA ANALYSIS: CLADISTICS

We used two subsets of characters that represented all (26 characters, data set 1), or only those characters lacking division into ranges of states (15 characters, data set 2) (Tables 3, 4). Parsimony analyses were conducted with PAUP* 4.0d64 (Swofford, 1998). *Solanum juglandifolium*, *S. lycopersicoides*, *S. ochranthum*, and *S. sitiens* were used as outgroups based on Rick

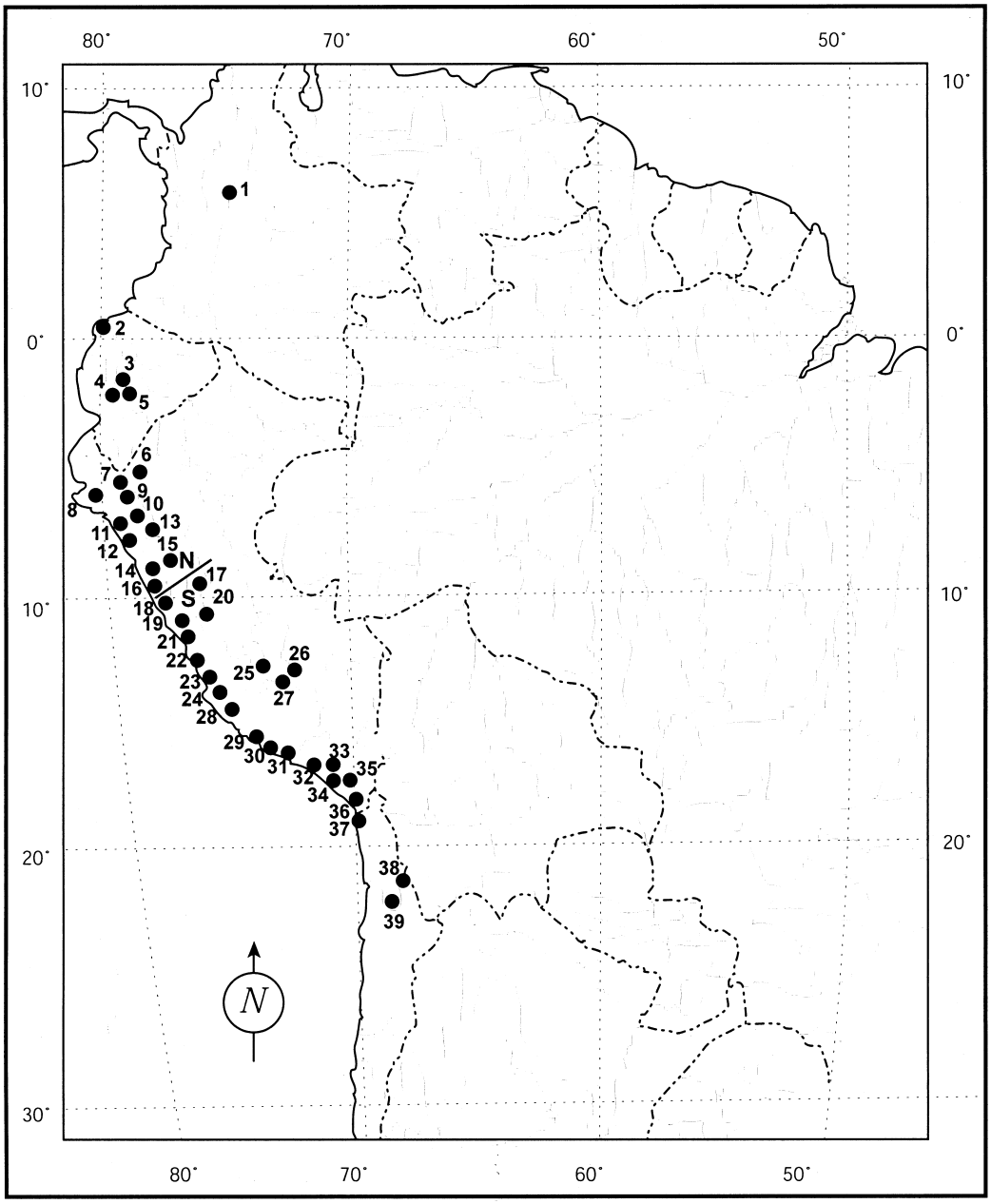

Figure 3. Distribution of the wild tomato accessions used in this study. Map numbers correspond to generalized map localities in Table 1. The line indicates the geographic distribution of *S. peruvianum* north (N) and south (S).

TABLE 3.

Morphological characters used in cladistic analyses of wild tomatoes and outgroups. All 26 characters are used in data set 1; those marked with an asterisk (*) are eliminated for data set 2 containing 15 characters. Consistency Index (CI) and Retention Index (RI) were estimated for each character. The RI is not applicable (n.a.) for autapomorphic characters.

1.* Number of nodes before branch bifurcation: 1, > 30; 2, 17–20; 3, 10–15; 4, < 10. CI = 1, RI = 1.
2. Number of leaves per sympodium: 1, > 5; 2, 3; 3, 2. CI = 0.667, RI = 0.857.
3. Pseudostipular leaves: 1, absent; 2, present. CI = 1, RI = 1.
4.* Number of lateral leaflet pairs: 1, 4–5; 2, 2–4. CI = 0.5, RI = 0.5.
5.* Number of secondary leaflets: 1, 0–5; 2, 5–10; 3, 10–20; 4, 40–50. CI = 1, RI = 1.
6. Tertiary leaflets: 1, absent; 2, present. CI = 1, RI = n.a.
7.* Leaflet width 5 mm below the apex: 1, > 15 mm; 2, < 10 mm. CI = 1, RI = n.a.
8.* Inflorescence peduncle length: 1, > 7 cm; 2, 5–7 cm; 3, 3–5; 4, < 3 cm. CI = 0.750, RI = 0.833.
9.* Branches per inflorescence: 1, > 4; 2, 2 often 3; 3, 1–2; 4, 1. CI = 1, RI = 1.
10. Bracts in inflorescence: 1, absent; 2, present. CI = 0.5, RI = 0.8.
11. Anthoclades (see Spooner et al., 1993): 1, 3.7; 2, 3.6. CI = 1, RI = 1.
12.* Pedicel articulation: 1, mid to upper position; 2, variable; 3, basal. CI = 1, RI = 1.
13. Corolla shape: 1, symmetrical; 2, asymmetrical. CI = 1, RI = n.a.
14.* Length from center of corolla to apex of corolla lobe: 1, >1.5 cm; 2, 1–1.5 cm; 3, < 1 cm. CI = 0.667, RI = 0.667.
15. Anther color: 1, white; 2, yellow. CI = 1, RI = 1.
16. Anther lateral interlocking papillae: 1, absent; 2, small; 3, elongate. CI = 1, RI = 1.
17. Anther connation: 1, separate; 2, free but holding together; 3, tightly connate. CI = 1, RI = 1.
18. Anther appendages: 1, absent; 2, present. CI = 1, RI = 1.
19. Anther tube: 1, straight; 2, anther and appendages curved; 3, anther curved. CI = 1, RI = n.a.
20.* Style exsertion: 1, > 1.8; 2, 1–1.8; 3, 0.5–1; 4, 0.5–0. CI = 0.750, RI = 0.857.
21. Fruit color: 1, green; 2, yellow to orange; 3, red. CI = 1, RI = 1.
22.* Fruit size: 1, > 25 mm diam. at widest point; 2, 15–25 mm diam. at widest point; 3, 5–15 mm diam. at widest point; 4, < 5 mm diam. at widest point. CI = 1, RI = 1.
23. Fruit pericarp: 1, thick and hard pericarp; 2, thin and leathery; 3, thin and membranaceous. CI = 1, RI = 1.
24. * Seeds: 1, more than 3 mm; 2, less than 3 mm. CI = 1, RI = 1.
25. Compatibility: 1, self-incompatible; 2, self-compatible. CI = 1, RI = 1.
26. Breeding: 1, allogamous; 2, autogamous/facultative allogamous; 3, autogamous. CI = 0.667, RI = 0.750.

TABLE 4. Data matrix of cladistic character states corresponding to Table 3.

		Characters and Character States																								
	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
OUTGROUPS	*Solanum lycopersicoides*	1	1	2	1	1	1	2	1	1	2	1	1	1	2	1	1	1	1	1	1	1	3	2	1	1	1
	S. sitiens	?	1	2	1	1	1	2	1	1	2	1	1	1	2	1	1	1	1	1	1	1	3	2	1	1	1
	S. ochranthum	1	1	2	2	1	1	2	2	1	1	2	2	1	2	2	2	2	1	1	1	1	1	1	1	1	1
	S. juglandifolium	1	1	2	2	1	1	2	2	1	1	2	2	1	2	2	2	2	1	1	1	1	1	1	1	1	1
INGROUPS	*S. pennellii*	3	3	2	2	1	1	1	2	3	2	1	3	2	1	2	2	3	1	3	1	1	3	3	2	1	1
	S. habrochaites	2	2	2	2	1	1	2	3	3	2	1	1	1	1	2	3	3	2	1	2	1	3	3	2	1	1
	S. chilense	3	3	2	1	1	1	2	1	2	2	1	1	1	1	2	3	3	2	1	2	1	3	3	2	1	1
	S. peruvianum south	3	3	2	2	1	1	2	2	2	2	1	1	1	1	2	3	3	2	2	2	1	3	3	2	1	1
	S. peruvianum north	3	3	2	2	1	1	2	2	4	2	1	1	1	2	2	3	3	2	1	3	1	3	3	2	1	1
	S. chmielewskii	3	3	2	2	1	1	2	4	4	2	1	1	1	2	2	3	3	2	1	3	1	3	3	2	2	2
	S. neorickii	3	3	2	2	1	1	2	4	4	2	1	1	1	3	2	3	3	2	1	4	1	3	3	2	2	3
	S. cheesmaniae	4	2	1	2	2	1	2	4	4	1	1	1	1	2	2	3	3	2	1	4	2	3	3	2	2	3
	S. galapagense	3	2	1	2	4	2	2	4	4	1	1	1	1	2	2	3	3	2	1	4	2	4	3	2	2	3
	S. pimpinellifolium	3	2	1	2	2	1	2	4	4	1	1	1	1	2	2	3	3	2	1	3	3	3	3	2	2	2
	S. lycopersicum	3	2	1	2	3	1	2	4	4	1	1	1	1	2	2	3	3	2	1	4	3	2	3	2	2	2

(1979, 1988), Child (1990), Spooner et al. (1993), and Peralta and Spooner (2001). *Solanum juglandifolium* and *S. ochranthum* are supported by GBSSI results as the sister taxa to tomatoes, but were not included in the phenetic analysis due to their slow growth in the greenhouse. We obtained flower, fruit, and seed cladistic characters of these species from the literature or from herbarium specimens.

Cladistic analyses were performed on both data sets 1 and 2, using Wagner parsimony, unordered character states, and all characters were equally weighted. The heuristic searches were performed using branch and bound and furthest addition sequence. The amount of homoplasy for both trees and individual characters was evaluated with the consistency index (CI) of Kluge and Farris (1969) and the retention index (RI) of Farris (1989). Consensus trees were generated from all most parsimonious trees. Bootstrap (Felsenstein, 1985) and Jackknife analyses, using 1000 replicates (using tree bisection reconnection and saving all multiple trees), were conducted to estimate the internal relative support for each branch.

RESULTS

CHARACTER STATE VARIATION

The Tukey-Kramer HSD test determined that 61 of the 66 characters were significantly different between at least two taxa (Table 2). The means, ranges, and standard deviations of the 30 characters showing most variation among species are presented in Figure 4. Separately, distribution of character states of "northern" and "southern" populations of *S. peruvianum*, chosen on the basis of the GBSSI results (Peralta & Spooner, 2001), were also compared using the Tukey-Kramer HSD test. Thirty-one characters were significantly different between these two groups of *S. peruvianum*. The means, ranges, and standard deviations of the 18 most important characters separating these geographic groups of *S. peruvianum* are illustrated in Figure 5.

PHENETIC RESULTS

The same dendrogram was produced by DIST (Fig. 6) and EUCLID, both with a cophenetic correlation coefficient of 0.93, which was only slightly higher than those produced by MANHAT, 0.91; CORR was 0.75. Cophenetic correlations between 0.8 and 0.9 can be interpreted subjectively as good fits to the cluster analysis, and those between 0.7 and 0.8 as poor fits (Rohlf, 1992).

The DIST phenogram has four main groups (Fig. 6A–D). The outgroups, *S. lycopersicoides* and *S. sitiens*, were clustered as the external branch (group D), followed by *S. galapagense*, and then a group of all three accessions of *S. pennellii* (group C). The self-compatible, red- to orange- to yellow-fruited species (*S. lycopersicum*, *S. cheesmaniae*, and *S. pimpinellifolium*) formed a third cluster (group A), but with the exclusion of the distinctive *S. galapagense*. The fourth group (B) included the remaining species.

Within group B, *Solanum neorickii* and two accessions of *S. chmielewskii* clustered together, to the exclusion of one accession of *S. chmielewskii* (LA 1306) that grouped with *S. peruvianum*. All accessions of *S. chilense* formed a group that also contained one accession of *S. peruvianum* (LA 1982). The three accessions of *S. habrochaites* formed a separate group. Concordant with the GBSSI sequence data, two major groups were recognized within *S. peruvianum*: "the northern" and "the southern" (Fig. 6 N, S). Only three *S. peruvianum* accessions were placed outside the northern and southern clusters (LA 2172, LA 1379, LA 1982).

We also present the CORR phenogram (Fig. 7) despite a lower cophenetic correlation (0.75; vs. DIST, 0.93), because it provides better phenetic support for *S. cheesmaniae* and *S. galapagense*, and north and south populations of *S. peruvianum*. Unlike the DIST phenogram, it places the two outgroups, *S. lycopersicoides* and *S. sitiens*, as an internal branch with one of two

main clusters (A). The self-compatible, red- to orange- to yellow-fruited species: *S. cheesmaniae*, *S. galapagense, S. lycopersicum*, and *S. pimpinellifolium* grouped together within the cluster A. The three accessions of *S. habrochaites* formed a separate group, and also the three *S. pennellii* accessions clustered together. The other main branch (B) includes *S. peruvianum*, *S. chilense*, *S. neorickii*, and *S. chmielewskii*. The CORR phenogram, unlike the DIST phenogram, showed a better clustering of the northern and southern *S. peruvianum* groups (Fig. 7), and maintained all accessions of *S. cheesmaniae* and *S. galapagense* together. Like the DIST phenogram, *S. peruvianum* (LA 1982) clustered with *S. chilense*. The two accessions of *S. peruvianum* f. *glandulosum* clustered with the southern *S. peruvianum*. *Solanum neorickii*, *S. chmielewskii*, and the two accessions of *S. peruvianum* var. *humifusum* clustered with the northern *S. peruvianum*.

The SDA of all taxa discriminated 30 characters. The 10 best characters, in decreasing order of discriminative utility, were: (1) anther attachment, (2) anther color, (3) pseudostipules, (4) number of nodes, (5) number of tertiary leaflets, (6) number of leaves per symposia, (7) trichome type III, (8) anther length, (9) plant height, and (10) number of secondary leaflets.

The SDA of the northern and southern populations of *S. peruvianum* discriminated 11 characters, (1) number of inflorescence branches, (2) style exsertion, (3) dorsal leaflet petiolule, (4) fruit color, (5) ratio: articulation length/pedicel length, (6) anther curvature, (7) internode length, (8) trichome type V, (9) pedicel length, (10) trichome type IV, and (11) terminal leaflet length.

CLADISTIC RESULTS

The analysis using all 26 characters (Tables 3, 4) produced 28 equally parsimonious trees, length 54 (Fig. 8). The strict consensus tree (Fig. 9) has a CI = 0.870, Consistency Index excluding uninformative characters (CIU) = 0.857, and RI = 0.900. Bootstrap analysis using 10,000 replicates gave 96% support for the ingroup. Jackknife values (10,000 replicates) were similar or sometimes lower, but also support these groups (Fig. 9). In the ingroup *Solanum pennellii* is supported as basal. The relationships among the self-incompatible species *S. chilense*, *S. habrochaites*, and *S. peruvianum* south were not resolved, and the three taxa formed a polytomy in all 28 equally parsimonious trees (Fig. 9). *Solanum peruvianum* north appeared as basal to *S. chmielewskii* and *S. neorickii* (Fig. 9) in the 28 equally parsimonious trees with 76(64)% bootstrap (jackknife) support. *Solanum chmielewskii* and *S. neorickii* appeared always together and supported in a basal position in the same clade as the monophyletic group formed by *S. cheesmaniae*, *S. galapagense*, *S. pimpinellifolium*, and *S. lycopersicum* (Fig. 9).

When the breeding characters (Table 3: 25, 26) were excluded, 24 trees were obtained (length = 50; CI = 0.880, CIU = 0867, RI = 0.902). A strict consensus tree of these 24 trees is identical to the strict consensus tree using all 26 characters (Fig. 9). The analysis using the subset of 15 characters (Table 3) produced 10 equally parsimonious trees, length 26. The strict consensus tree (CI = 0.846, CIU = 0.818, RI = 0.895) is topologically the same that was obtained using all 26 characters (Fig. 9), except *S. peruvianum* north is now part of the polytomy with *Solanum chilense*, *S. habrochaites*, and *S. peruvianum* south.

CONCORDANT MORPHOLOGICAL CLADISTIC RESULTS

The cladistic results showed the following patterns of relationships: (1) Within the four outgroup taxa, *Solanum ochranthum* and *S. juglandifolium* were sister taxa and *S. lycopersicoides* and *S. sitiens* were sister taxa. (2) Within the tomato ingroup, *S. pennellii* was basal. (3) *Solanum cheesmaniae, S. galapagense*, *S. pimpinellifolium*, and *S. lycopersicum* were terminal taxa and monophyletic. (4) *Solanum chmielewskii* and *S. neorickii* appeared always together in a basal position in the same clade as *S. cheesmaniae*, *S. galapagense, S. pimpinelli-*

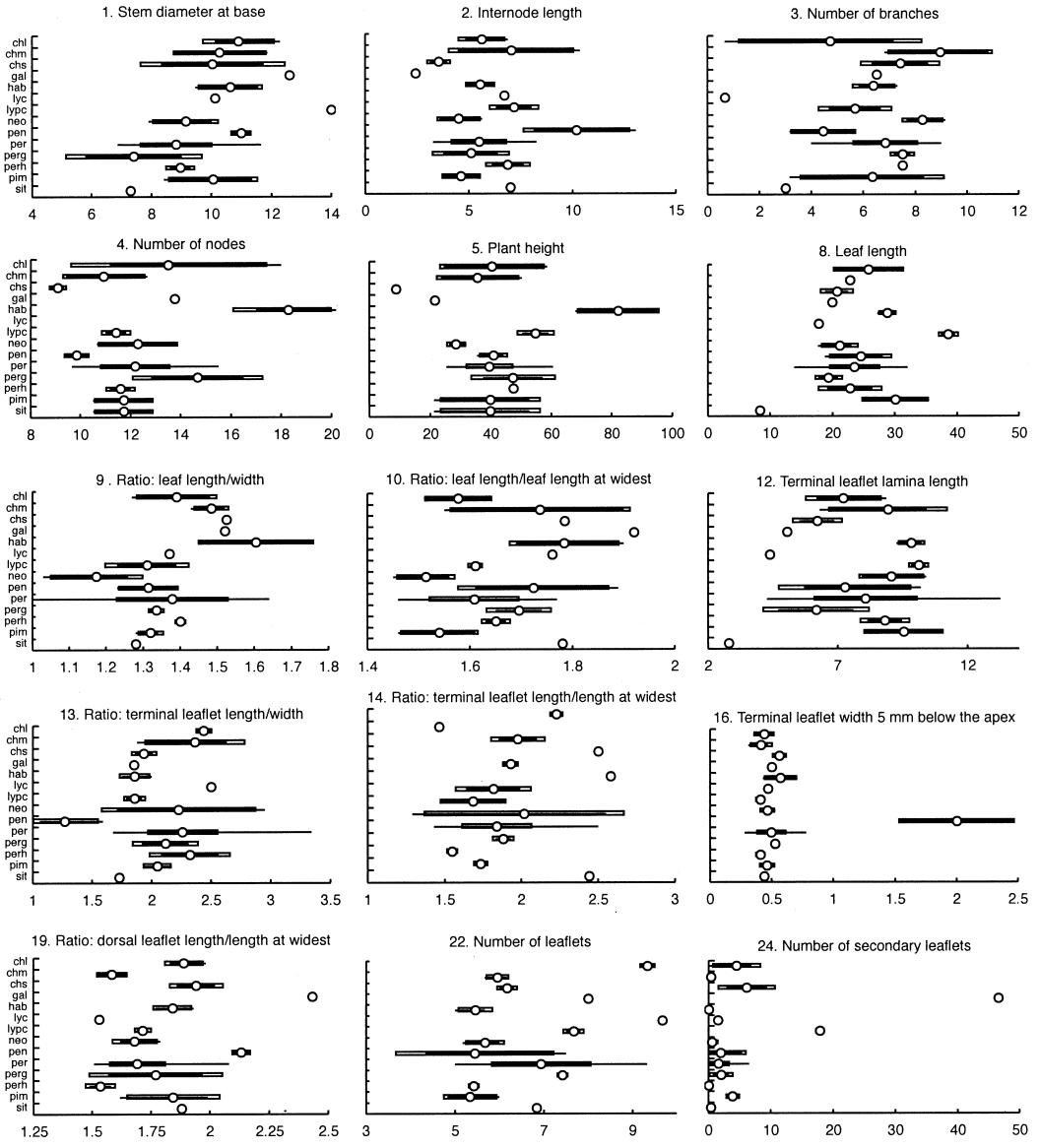

Figure 4. Means, ranges, and one standard deviation of the mean for the 30 of 66 characters examined in this study showing the greatest differences among taxa of *Solanum chilense* = chl; *S. chmielewskii* = chm; *S. cheesmaniae* = chs; *S. galapagense* = gal [= *S. cheesmaniae* f. *minor*]; *S. habrochaites* = hab; *S. lycopersicoides* = lyc; *S. lycopersicum* var. *cerasiforme* = lypc; *S. neorickii* = neo; *S. pennellii* = pen; *S. peruvianum* = per; *S. peruvianum* f. *glandulosum* = perg; *S. peruvianum* var. *humisufum* = perh; *S. pimpinellifolium* = pim; *S. sitiens* = sit.

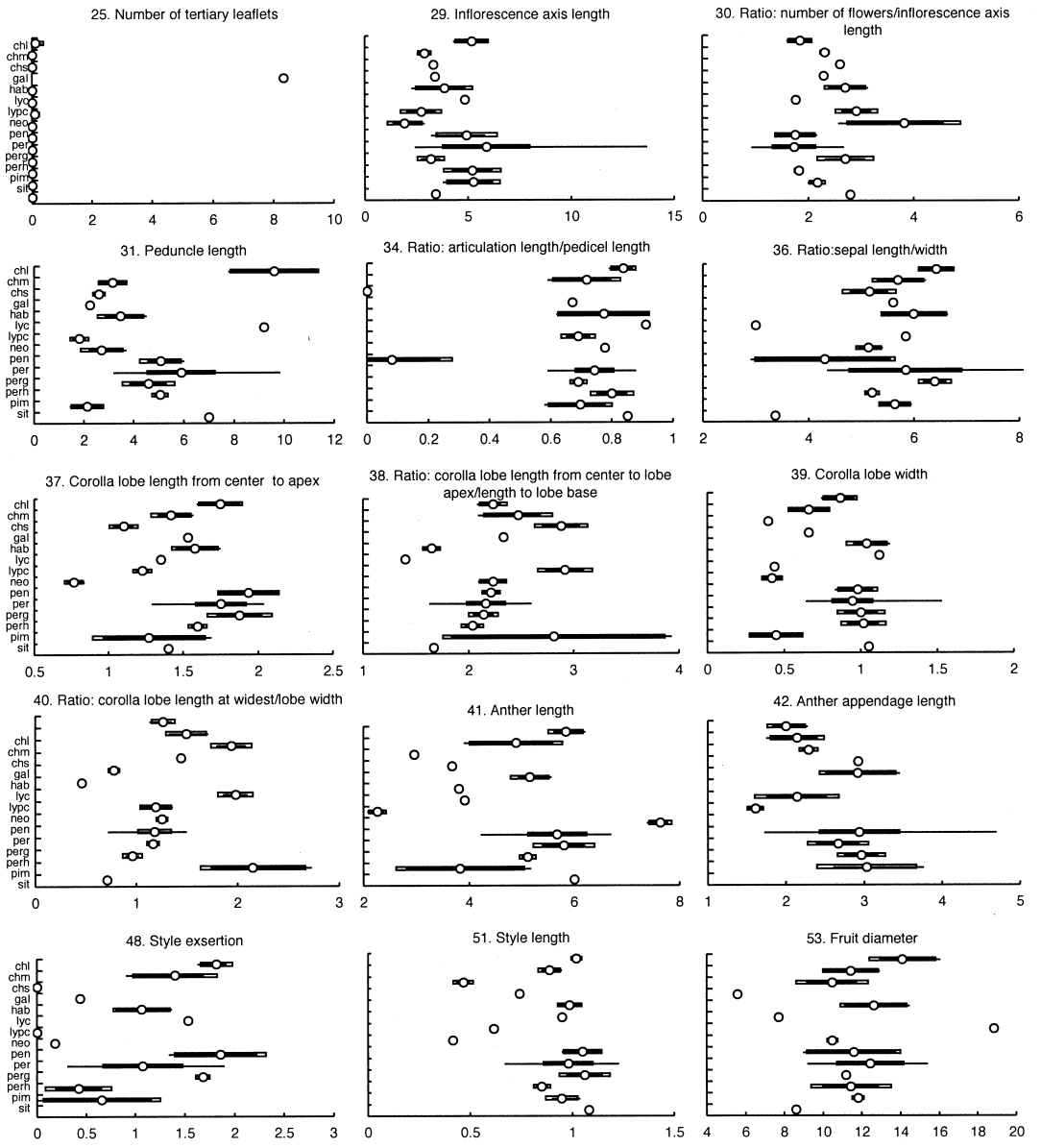

Figure 4 continued.

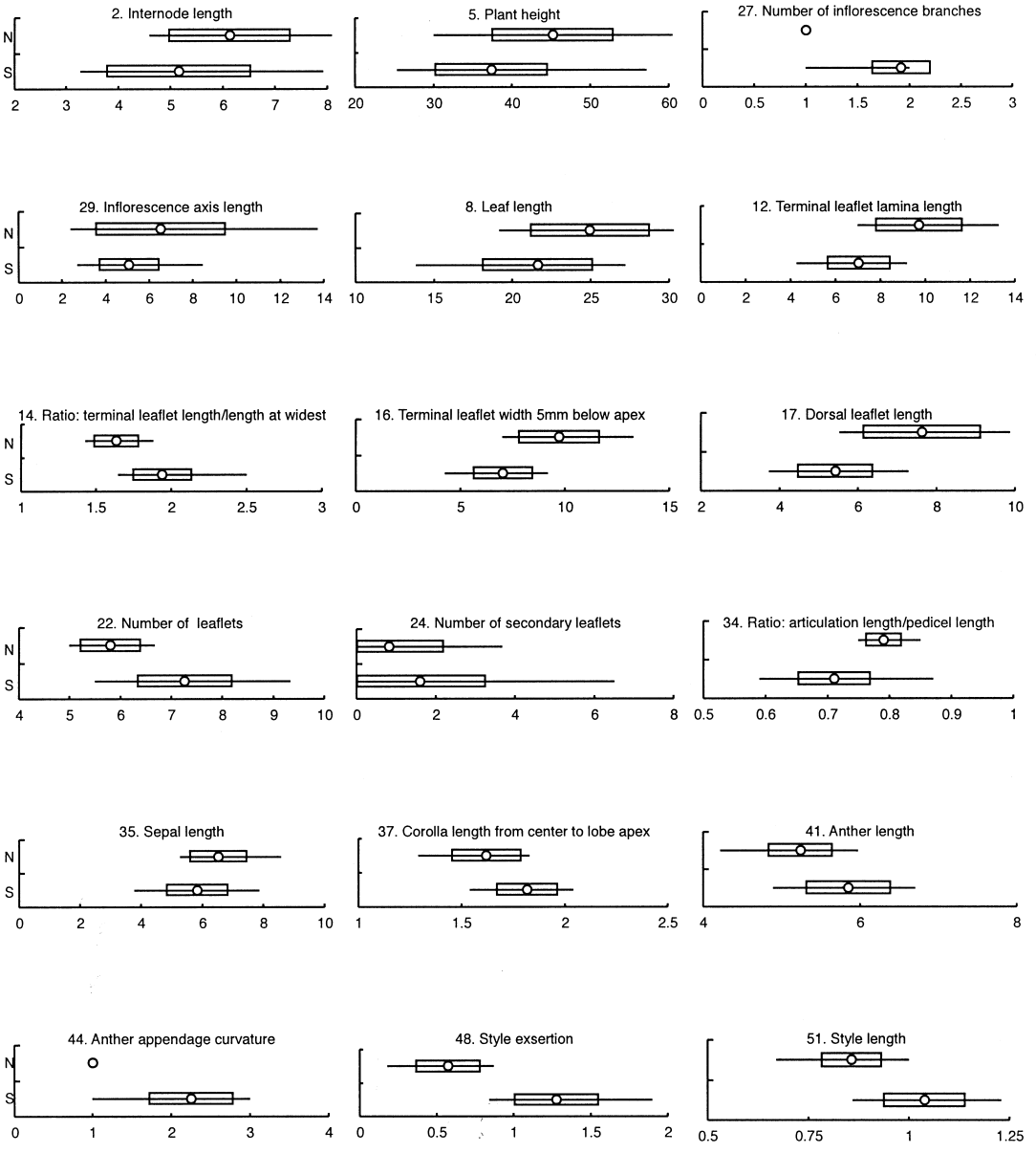

Figure 5. Means, ranges, and one standard deviation of the mean for the 18 of 61 characters examined in this study showing the greatest differences among *S. peruvianum* accessions from northern Peru = N, and southern Peru = S.

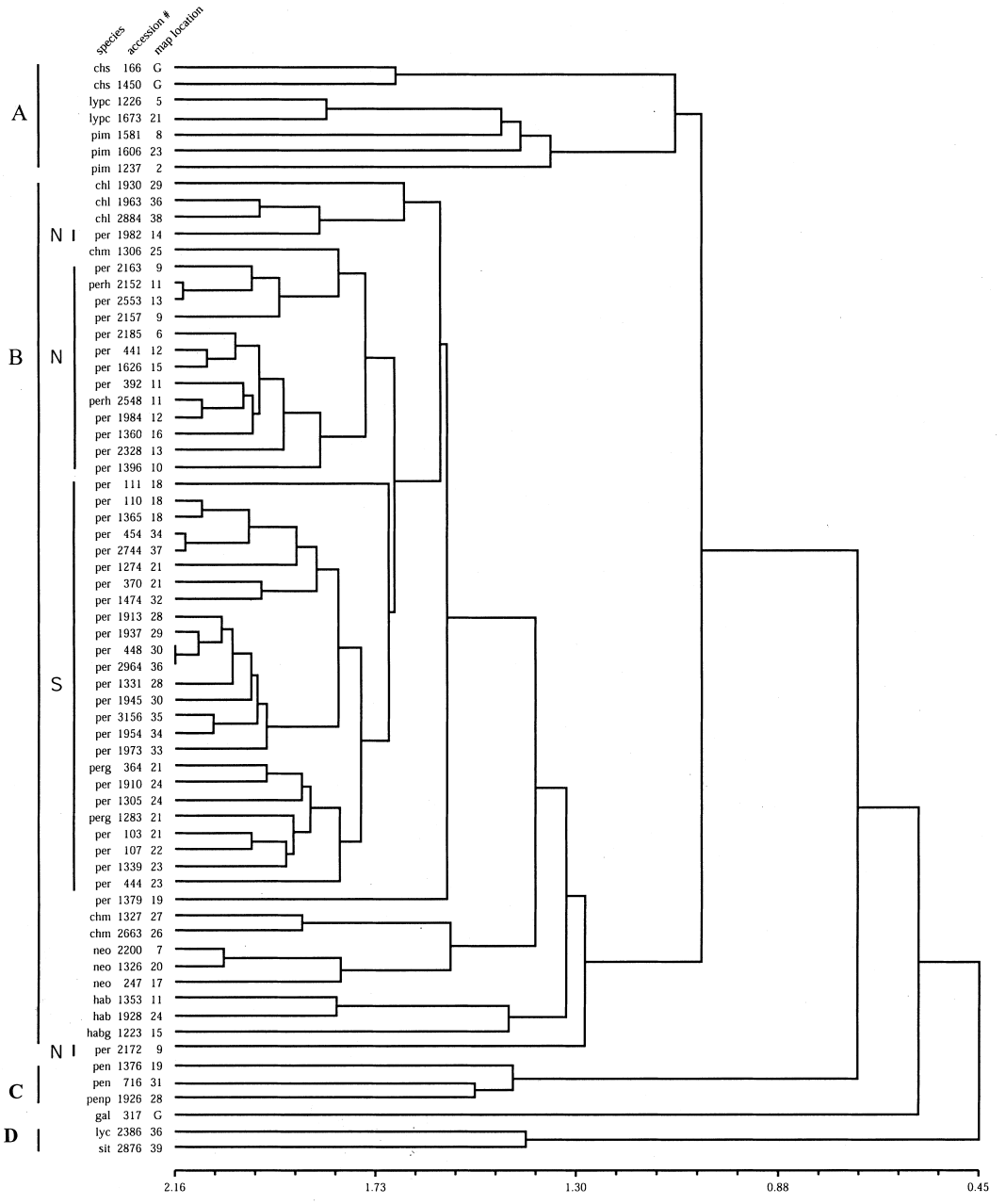

Figure 6. UPGMA dendrogram (DIST similarity option) based on 61 morphological characters. Species codes, accession numbers, and map locations as in Table 1.

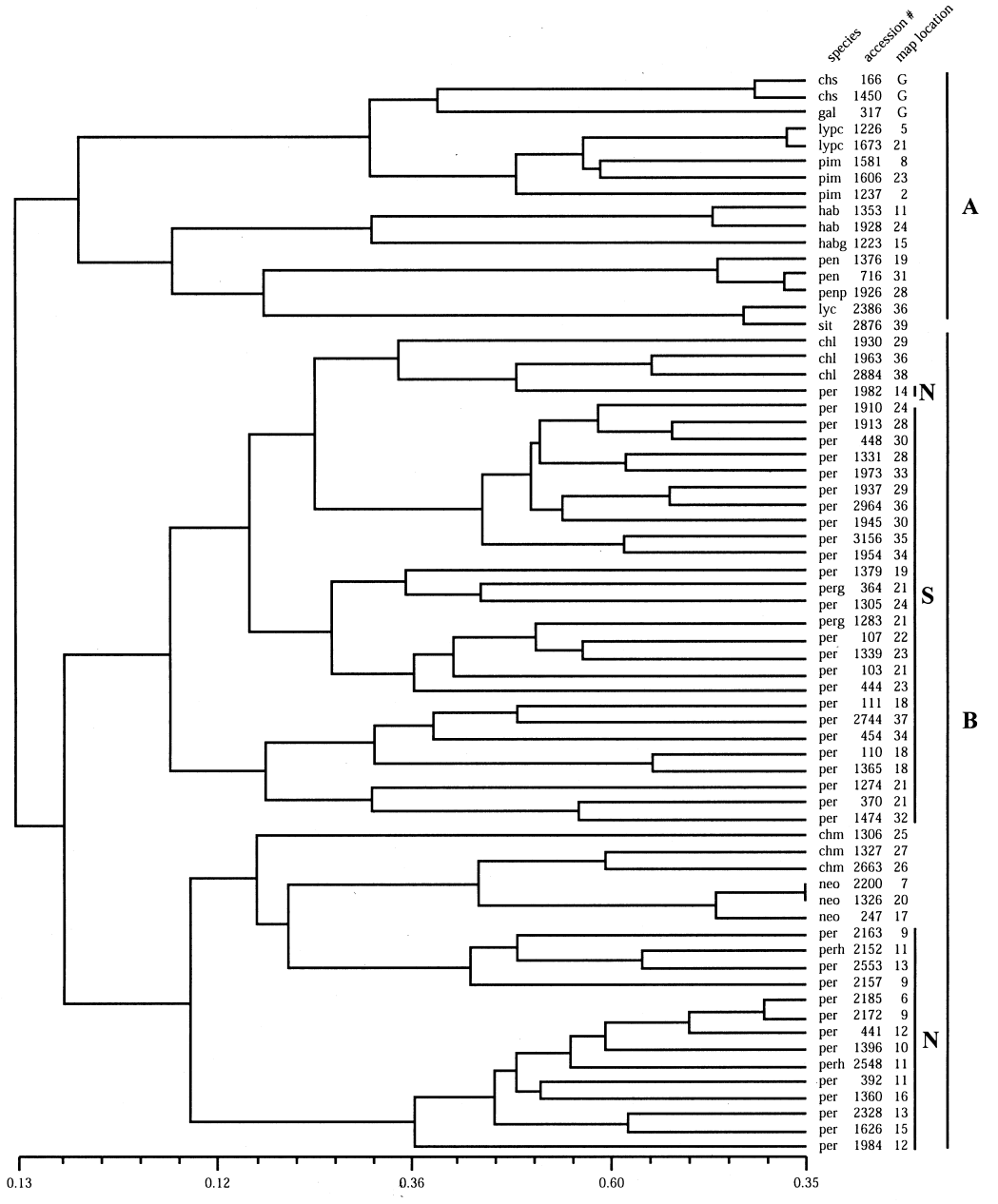

Figure 7. UPGMA dendrogram (CORR similarity option) based on 61 morphological characters. Species codes, accession numbers, and map locations as in Table 1.

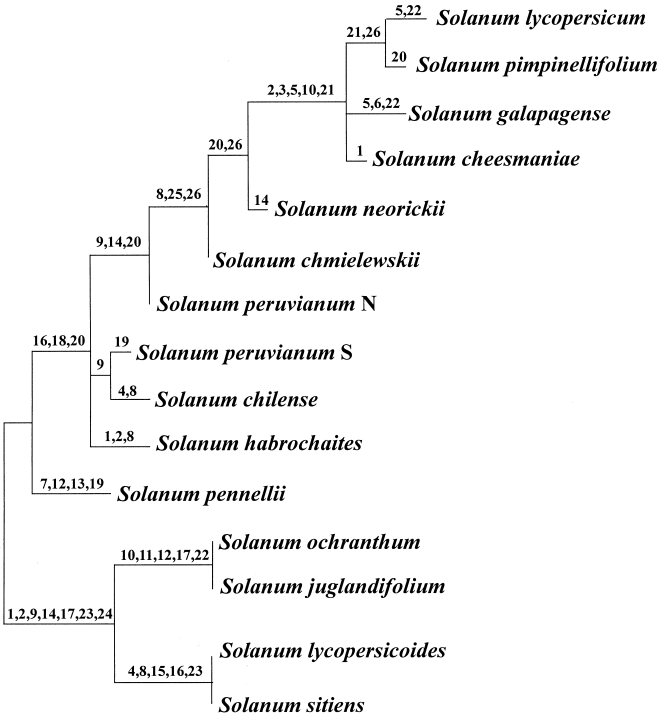

Figure 8. One representative phylogram of 28 equally parsimonious trees, length = 54, based on all 26 unordered morphological characters. CI = 0.870, CIU = 0.857, RI = 0.900.

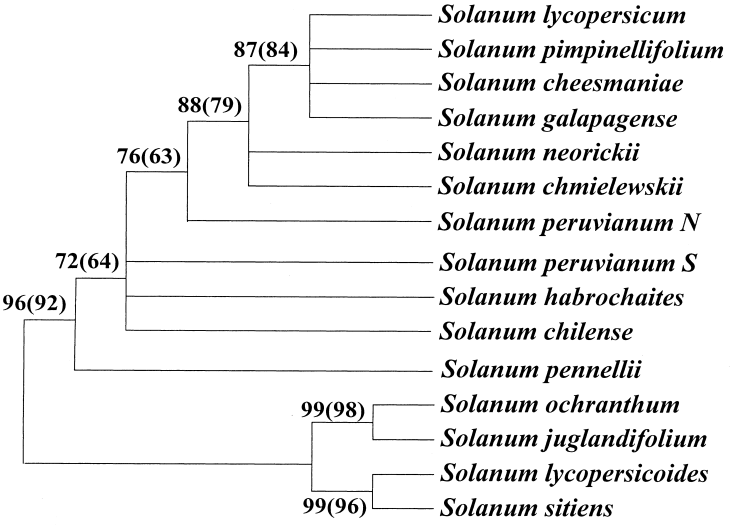

Figure 9. Strict consensus tree of the 28 most parsimonious trees (length 54) based on all 26 unordered morphological characters. Percentage of 10,000 bootstrap replicates, followed by percentage of 10,000 jackknife replicates (in parentheses), is given above branches. The same strict consensus topology was obtained in the analysis excluding the two breeding characters, and nearly the same topology (except for *S. peruvianum* N that was part of the basal polytomy with *S. peruvianum* S, *S. habrochaites,* and *S. chilense*) in the analysis of 15 characters (25, 26, Tables 3, 4).

folium, and *S. lycopersicum*. (5) The self-incompatible *S. chilense*, *S. habrochaites*, and *S. peruvianum* south form a basal polytomy in the ingroup (the 15-character data set included *S. peruvianum* north in this basal polytomy).

CONCORDANCE OF MORPHOLOGICAL PHENETICS AND CLADISTICS

The phenetic results are not designed to show phylogenetic relationships. When similarities due to shared ancestral characteristics or homoplasy exceed similarities due to shared derived characteristics, the phenogram will not represent the true phylogeny, but if there were no homoplasy, the phenogram constructed by the phenetic analysis would correspond to the true phylogenetic tree (Futuyma, 1998). The similarities of phenetic and cladistic results show: (1) The outgroup taxa were differentiated in both analyses. (2) *Solanum cheesmaniae*, *S. galapagense, S. pimpinellifolium*, and *S. lycopersicum* were also consistent "groups." (3) *Solanum peruvianum* was differentiated in two groups from northern and southern Peru, with 76% bootstrap support in the cladistic results (Fig. 9). (4) *Solanum chmielewskii* and *S. neorickii* grouped with the northern *S. peruvianum* but not with *S. cheesmaniae*, *S. galapagense, S. pimpinellifolium*, and *S. lycopersicum* as in the cladistic results. (5) *Solanum chilense* grouped with the southern *S. peruvianum* in the phenetic results (Fig. 7), but in the cladistic results *S. chilense* appeared as a basal polytomy with southern *S. peruvianum* and *S. habrochaites* (Fig. 9).

CONCORDANCE OF MORPHOLOGICAL CLADISTICS AND GBSSI CLADISTICS

There are striking concordances between the morphological cladistic results shown here and the GBSSI cladistic results of Peralta and Spooner (2001). (1) Within the four outgroup taxa, *Solanum ochranthum* and *S. juglandifolium* were sister taxa and *S. lycopersicoides* and *S. sitiens* were sister taxa. (2) Within the tomato ingroup, *S. pennellii* was supported as a basal taxon. (3) *Solanum habrochaites* also appeared in a basal position. (4) *Solanum peruvianum* south showed a close relation with the last two taxa and also with *S. chilense*. (5) *Solanum cheesmaniae*, *S. galapagense*, *S. pimpinellifolium*, and *S. lycopersicum* were terminal taxa and monophyletic. (6) *Solanum chmielewskii* and *S. neorickii* were basal to *S. cheesmaniae*, *S. galapagense, S. pimpinellifolium*, and *S. lycopersicum*. GBSSI and morphological results showed a closer relationship of these three last taxa with *S. chmielewskii* and *S. neorickii*, which are also related to the northern populations of *S. peruvianum*.

DISCUSSION

PHENETICS

The above analysis of character state variation and phenetic relationships provided useful data to (1) help support existing species, (2) support the northern and southern populations of *Solanum peruvianum* as separate taxa, (3) show character state variation useful for species descriptions, and (4) show character states useful for cladistic analyses.

Although the range of values for many characters did not show discrete breaks to differentiate species, some have breaks in variability useful for species differentiation and cladistic analysis. The most important morphological characters, determined by the simple statistics and SDA, agreed with those previously used in taxonomic diagnoses and construction of keys (Müller, 1940; Luckwill, 1943; Rick et al., 1977, 1990; Taylor, 1986). Qualitative flower character states, such as white, free anthers with large filaments, and more than five leaves per sympodium were only shared by the species of subsection *Lycopersicoides*. Development of pseudostipules and inflorescence bracts, number of inflorescence branches, anther curvature, color and pubescence of the fruits, and trichome types have taxonomic value within wild tomatoes. *Solanum pennellii* traditionally has been recognized by its particular anther structure, lacking the sterile tip, poricidal dehiscence, and slightly asymmetric flowers.

Although most of the quantitative characters evaluated had continuous distribution of states across taxa, some of them were useful for species diagnosis. Short plants with short internode lengths and early formation of inflorescence (8th to 9th node) characterize *Solanum cheesmaniae*, while in the shrubby, low-branched, and taller plants of *S. lycopersicoides*, inflorescences were generated after the 35th node. *Solanum sitiens* can be identified by its smaller and narrow leaflets. *Solanum neorickii* was recognized by its small flowers with short anther tips, short inflorescence axis, and a high number of flowers in relation to the axis length. Diagnostic characters for *S. chilense* were large peduncles, leaves with few secondary and tertiary leaflets, and large flowers and fruits. Taller, very robust, and pubescent plants characterized *S. habrochaites*, which produces inflorescences after the 18th node, and has leaves without secondary leaflets. In *S. pennellii*, the width of the leaflet lamina, as well as anther length and structure, are valuable diagnostic characters. *Solanum lycopersicum* produced the largest fruits and numerous secondary leaflets, while *S. pimpinellifolium* presented the largest ratio between corolla lobe length and corolla lobe width, indicating a stellate shape.

The phenetic analysis distinguished the "northern" and "southern" groups in *S. peruvianum*. This result was postulated by morphology and crossing relationships (Rick, 1963, 1979, 1986b), and was also supported by the GBSSI phylogeny on the same accessions (Peralta & Spooner, 2001). The SDA selected similar sets of characters to those used previously to characterize the northern races, such as unbranched inflorescence, straight anther tubes, reduced style exsertion, and 5 to 7 leaflets (Rick, 1986b). Although most of the quantitative characters had a continuous distribution of states across these northern and southern populations, some of them (plant height, internode length, inflorescence axis length, leaf length, size of leaflets, and sepal length) showed that taller and more robust plants characterized the northern accessions. In contrast, the southern accessions can be differentiated by the presence of few secondary leaflets, branched inflorescences, bright yellow larger corollas, bright yellow larger and curved anthers, and exserted styles.

CLADISTICS

Concordance among phenetic and cladistic results might indicate that the amount of homoplasy does not outnumber similarities due to shared derived characteristics in wild tomatoes. The following discussion focuses on cladistic results of morphological, GBSSI, and other molecular data to comment on prior phylogenetic hypotheses in tomatoes (above). Cladistic results of morphological features confirm ingroup relationships shown by nuclear RFLP data (Miller & Tanksley, 1990), cpDNA restriction site data (Palmer & Zamir, 1982), and GBSSI DNA sequence data (Peralta & Spooner, 2001). Phenetic and cladistic analyses of morphological characters showed the distinct nature of the northern and southern Peruvian populations of *Solanum peruvianum*, a result consistent with GBSSI sequence data. The morphology shown here, as well as the nuclear RFLP (Miller & Tanksley, 1990), cpDNA restriction site (Palmer & Zamir, 1982), and GBSSI data (Peralta & Spooner, 2001), do not agree with the mitochondrial DNA phenetic results (McClean & Hanson, 1986).

Monophyly of the self-compatible species with pigmented fruits, *Solanum cheesmaniae, S. galapagense*, *S. pimpinellifolium*, and *S. lycopersicum*, has been shown by the cpDNA phylogeny, GBSSI data, and nuclear RFLP data, and here confirmed by morphology. Furthermore, *S. neorickii* and *S. chmielewskii* were placed with northern races of *S. peruvianum* as recently shown by cpDNA, nuclear RFLP, and GBSSI data. *Solanum chmielewskii* and *S. neorickii* appeared supported together in a basal position in the same clade as *S. cheesmaniae*, *S. galapagense, S. pimpinellifolium*, and *S. lycopersicum*. Our morphological and GBSSI results showed the northern accessions of *S. peruvianum* as closely related to *S. neorickii*, *S. chmielewskii*, and the three red- to orange- to yellow-fruited species of the Esculentum complex, discordant with Rick's (1979) classification (Fig. 1). Furthermore, the

morphological results, as well as cpDNA, nuclear RFLP, and GBSSI data, are discordant with the placement of *S. pennellii* and *S. habrochaites* in the Esculentum group (Rick, 1979). On the other hand, *Solanum chilense* showed close relationships with the southern accessions of *S. peruvianum*, as indicated by crossability (Rick, 1979), cpDNA, and GBSSI phylogeny.

Morphological and molecular data support allogamy, self-incompatibility, and green fruits as plesiomorphic in tomatoes. The basal taxa also share characters such as branched and bracted inflorescences and leaves usually with pseudostipules. These results do not support the phylogenetic hypothesis proposed by Luckwill (1943), where all tomato species evolved from an ancestral stock with unbranched inflorescences, leaves with few leaflets, and without pseudostipules.

Large and attractive flowers have also been associated with the presence of a self-incompatibility system, and with the extent of outcrossing and genetic variation. Rick (1982b) postulated that three independent genetic systems are operating on the prevention of self-fertilization, changes in flower parts to ensure cross pollination, and development of secondary flower display characters to attract pollinators. Interestingly, a recent linkage analysis and quantitative trait loci (QTL) mapping of reproductive behavior and floral traits in wild tomatoes showed that major QTLs for several characters important to pollination biology harbor in the same region of the self-incompatibility locus, S, on chromosome 1. These QTL results suggest that a gene complex is controlling both genetic and morphological mechanisms of reproduction, and this gene complex may have been conserved since early periods of plant evolution or may reflect a convergent evolutionary process (Bernacchi & Tanksley, 1997).

Several characters of the corolla, anthers, and stigma (Table 2), associated with floral display, pollinator attraction, and reproduction, were included in this morphological study. The self-incompatible accessions of *S. habrochaites* and *S. pennellii* (Table 1) showed larger corollas and anthers as well as more exserted stigmas than the self-compatible accessions. Similarly, in the self-compatible *S. pimpinellifolium* one facultative allogamous accession has larger flower parts and stigma exsertion than the two autogamous accessions. Changes in flower size and stigma exsertion also occur in the two sibling self-compatible species, *S. chmielewskii* and *S. neorickii*. The accessions of the first species showed larger flower parts, while the accessions of the exclusively autogamous *S. neorickii* have smaller flower parts and stigmas included in the anther tube. The southern self-incompatible accessions of *S. peruvianum*, like the outcrossing genotypes of *S. habrochaites*, *S. pennellii*, and *S. pimpinellifolium*, also displayed floral traits for pollination attraction. Larger, bright yellow corollas, larger, curved and asymmetrical anthers, and exserted styles are features that probably correlate with the high outcrossing rates found in *S. peruvianum* (Rick et al., 1977). Actually, branched inflorescences with many showy open flowers (usually 8 to 10) act as the attractive unit for pollinators (Rick et al., 1978). In contrast, the northern *S. peruvianum* accessions have unbranched inflorescences, smaller corollas, short and straight anther tubes, and reduced stigma exsertion. The differences found in inflorescence and flower morphology, and crossing behavior between northern and southern *S. peruvianum* races, raise an interesting question about the role that environmental factors might play in such differentiation. Northern races are widespread from the coast to interior uplands in the Andes (north of 8°S) in more mesic-subtropical habitats (Rick, 1986b). In contrast, the southern races are distributed along the arid Pacific coast (south of 8°S) to northern Chile, and in the adjacent Andean Mountains (Rick, 1986b). The present hyper-arid climates between 5°S and 28°S result primarily from the rain shadow effects of the Andes and the subtropical convergence of winds, and the drying effects of the cold Humboldt Current. Paleobotanical, paleonto-

logical, and geological evidences suggest that such harsh climatic conditions developed recently during the Holocene (Arroyo et al., 1988). The Neotropical plant communities that occupied the area before the climatic change were displaced toward more southerly and boreal latitudes, and a new flora became adapted to the new xeric conditions (Villagran et al., 1983). A similar scenario can be hypothesized to explain the differentiation between *S. peruvianum* groups. The southern populations became adapted to the unstable, very arid conditions of the coastal Pacific range. Under such harsh climatic conditions, lower pollinator availability and an increase of anemophily in the plant communities have been reported (Arroyo et al., 1983). Selection pressures to ensure crossing in self-incompatible plants may have driven changes in flower traits that increase insect attraction. The shifts of secondary flower characters are correlated with increase in levels of heterozygosity in populations (Rick et al., 1977). An opposite change toward reduction of flower organs and attractiveness has been reported in the self-compatible *S. cheesmaniae*. This endemic species of the Galápagos Islands grows in isolated, unstable, and dry habitats, where very few pollinator species are found. The *S. cheesmaniae* populations evolved toward strict autogamy, and genetic variation was only found between populations (Rick, 1986b).

Genetic mechanisms ensuring crossing may have played a role to maintain gene flow among southern *S. peruvianum* populations, as has been supported by hybridization studies (Rick, 1963, 1979, 1986b). In contrast, the northern populations growing in more stable habitats developed isolation mechanisms that prevent gene flow in sympatric populations. In addition to the prezygotic barriers, postzygotic isolating mechanisms have been found in this group (Ehlenfeldt & Hanneman, 1992).

A possible scenario in the evolution of wild tomatoes can be hypothesized taking into account the adaptation to changing environments, as well as the interaction with biotic factors such as availability of pollinators. The shift toward compatibility and reduction of floral structures also played an important role in the speciation process. Putative SI ancestral populations probably occupied a wide area of distribution in central Peru. After the drastic climatic change during the Holocene and the formation of the Peruvian–Chilean desert, a process of adaptation to the new habitats may have occurred leading to differentiation and speciation. Some populations, adapted to more mesic and humid conditions, colonized the northern areas. Changes from SI to SC may have originated autogamous populations, further accentuating isolation and probably genetic drift, and contributed to differentiation and speciation. Putative ancestors from the Rio Marañón area may have originated self-compatible taxa, as suggested by Rick (1986b). On the other hand, populations growing in arid habitats may have differentiated and migrated following the Pacific coast and colonized the adjacent western slopes, but were limited in their altitudinal distribution by frost. Selection pressure to ensure crossing in the SI populations may have driven evolutionary changes, and genetic differentiation most probably occurred by gene substitution (Rick, 1986b). Reproductive isolating barriers arose to prevent gene flow between sympatric populations. Analogously, putative ancestors of *S. juglandifolium* and *S. ochranthum* became adapted to more wet and warm environments, while ancestors of *S. sitiens* and *S. lycopersicoides* evolved in the desert.

Some taxonomic problems remain, and more studies are needed to elucidate the relationships between *Solanum chmielewskii* and the northern *S. peruvianum* races, and between *S. chilense* and the southern *S. peruvianum* races. Palmer and Zamir (1982), based on a cpDNA phylogeny, suggested that *S. chmielewskii* and *S. chilense* could be considered at the subspecific level within *S. peruvianum*. Müller (1940) considered *S. chilense* as a variety of *S. peruvianum*, while Luckwill (1943) included it as the subspecies *puberulum*. Our morphological results,

based on few *S. chmielewskii* and *S. chilense* accessions, provided good diagnostic characters. Nevertheless, analysis of more collections and herbarium specimens, especially those from original types, will help to clarify taxonomic problems. Studies of *S. peruvianum* infraspecific categories as well as the species recognized as *L. glandulosum* and *L. pissisi* are currently in progress. Rick and Lamm (1955), based on the photograph of the original collection of *L. pissisi*, concluded that this specimen was not related to *S. peruvianum*. Nevertheless, the identity of *L. pissisi* remains unsolved.

Our morphological results support existing species and support the northern and southern populations of *S. peruvianum* as separate taxa that may represent two distinct species.

Morphological data showed valuable diagnostic characters for species differentiation and phylogenetic relationships in wild tomatoes. These results are currently being used to prepare a monographic study of *Solanum* sections *Lycopersicon, Lycopersicoides,* and *Juglandifolium.* This monograph will include a new classification based on a synthesis of different data sets, reflecting the phylogenetic relationships of wild tomato species.

NOTE IN PROOF

This paper showed the need to reconsider the species limits of *Solanum peruvianum;* this was accomplished by Peralta et al. (2005) who recognized four species within *Solanum peruvianum* sensu lato, necessitating the publication of two new names.

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LITERATURE CITED

Arroyo, M. T. K., J. J. Armesto & R. Primack. 1983. Tendencias altitudinales y latitudinales en mecanismos de polinización en la zona andina de los andes templados de Sudamérica. Revista Chilena Hist. Nat. 56: 159–180.

—, F. A. Squeo, J. J. Armesto & C. Villagran. 1988. Effects of aridity on plant diversity in the northern Chilean Andes: Results of a natural experiment. Ann. Missouri Bot. Gard. 75: 55–78.

Bernacchi, D. & S. D. Tanksley. 1997. An interspecific backcross of *Lycopersicon esculentum* × *L. hirsutum*: Linkage analysis and a QTL study of sexual compatibility factors and floral traits. Genetics 147: 861–877.

Bohs, L. & R. G. Olmstead. 1997. Phylogenetic relationships in *Solanum* (Solanaceae) based on *ndh*F sequences. Syst. Bot. 22: 5–17.

— & —. 1999. *Solanum* phylogeny inferred from chloroplast DNA sequence data. Pp. 97–110 *in* M. Nee, D. E. Symon, R. N. Lester & J. P. Jessop (editors), Solanaceae IV, Advances in Biology and Utilization. Royal Botanic Gardens, Kew.

Child, A. 1990. A synopsis of *Solanum* subgenus *Potatoe* (G. Don) D'Arcy [*Tuberarium* (Dun.) Bitter (s.l.)]. Feddes Repert. 101: 209–235.

Correll, D. S. 1958. A new species and some nomenclatural changes in *Solanum* section *Tuberarium*. Madroño 14: 232–236.

Darwin, S. C., S. Knapp & I. E. Peralta. 2003. Taxonomy of tomatoes in the Galápagos Islands: Native and introduced species of *Solanum* section *Lycopersicon* (Solanaceae). Syst. Biodiversity 12: 29–53.

D'Arcy, W. G. 1972. Solanaceae studies II: Typifications of subdivisions of *Solanum* section *Tuberarium*. Ann. Missouri Bot. Gard. 59: 262–278.

Dunal, M. F. 1852. Solanaceae. Pp. 1–690 *in* A. L. P. P. de Candolle (editor), Prodromus systematis naturalis regni vegetalis 13. Treuttel and Würtz, London.

Ehlenfeldt, M. K. & R. E. Hanneman Jr. 1992. Endosperm dosage relationships among *Lycopersicon* species. Theor. Appl. Genet. 83: 367–372.

Esquinas Alcazar, J. T. 1981. Genetic resources of tomatoes and wild relatives. Rep. Int. Board Pl. Genet. Res. No. AGP. IBPGR/80/103: 1–65. Rome.

Farris, J. S. 1989. The retention index and homoplasy excess. Syst. Zool. 38: 406–407.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using bootstrap. Evolution 39: 783–791.

Futuyma, D. J. 1998. Evolutionary Biology, 3rd ed. Sinauer, Sunderland, Massachusetts.

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.

Hay, A., J. Craft & M. Tsiantis. 2004. Plant hormones and homeoboxes: Bridging the gap? BioEssays 26.4: 395–404.

Johnston, S. A., T. P. M. Den Nijs, S. J. Peloquin & R. E. Hanneman Jr. 1980. The significance of genetic balance to endosperm development in interspecific crosses. Theor. Appl. Genet. 53: 262–269.

Kluge, A. G. & J. S. Farris. 1969. Qualitative phyletics and the evolution of anurans. Syst. Zool. 18: 1–32.

Laterrot, H. 1989. The tomato. Advantages and use of wild varieties for varietal creation. Rev. Hort. 295: 13–17.

Luckwill, L. C. 1943. The genus *Lycopersicon*: An historical, biological, and taxonomical survey of the wild and cultivated tomatoes. Aberdeen Univ. Stud. 120: 1–44.

McClean, P. E. & M. R. Hanson. 1986. Mitochondrial DNA sequence divergence among *Lycopersicon* and related *Solanum* species. Genetics 112: 649–667.

Miller, J. C. & S. D. Tanksley. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theor. Appl. Genet. 80: 437–448.

Müller, C. H. 1940. A revision of the genus *Lycopersicon*. U.S.D.A. Misc. Publ. 382: 1–28 + 10 pl.

Olmstead, R. G. & J. D. Palmer. 1997. Implications for phylogeny, classification, and biogeography of *Solanum* from cpDNA restriction site variation. Syst. Bot. 22: 19–29.

—, J. A. Sweere, R. E. Spangler, L. Bohs & J. D. Palmer. 1999. Phylogeny and provisional classification of the Solanaceae based on chloroplast DNA. Pp. 111–137 *in* M. Nee, D. E. Symon, R. N. Lester & J. P. Jessop (editors), Solanaceae IV, Advances in Biology and Utilization. Royal Botanic Gardens, Kew.

Ortiz, R. & M. K. Ehlenfeldt. 1992. The importance of Endosperm Balance Number in potato breeding and the evolution of tuber-bearing *Solanum* species. Euphytica 60: 105–113.

Palmer, J. D. & D. Zamir. 1982. Chloroplast DNA evolution and phylogenetic relationships in *Lycopersicon*. Proc. Natl. Acad. Sci. U.S.A. 79: 5006–5010.

Peralta, I. E. & D. M. Spooner. 2000. Classification of wild tomatoes: A review. Kurtziana 28: 45–54.

— & —. 2001. GBSSI gene phylogeny of wild tomatoes (*Solanum* L. section *Lycopersicon* [Mill.] Wettst. subsection *Lycopersicon*). Amer. J. Bot. 88: 1888–1902.

—, S. K. Knapp & D. M. Spooner. 2005. New species of wild tomatoes (*Solanum* section *Lycopersicon:* Solanaceae) from northern Peru. Syst. Bot.: in press.

Rick, C. M. 1960. Hybridization between *Lycopersicon esculentum* and *Solanum pennellii*. Phylogenetic and cytogenetic significance. Proc. Natl. Acad. Sci. U.S.A. 46: 78–82.

—. 1963. Barriers to interbreeding in *Lycopersicon peruvianum*. Evolution 17: 216–232.

—. 1973. Potential genetic resources in tomato species: Clues from observations in native habitats. Pp. 255–269 *in* A. M. Srb (editor), Genes, Enzymes, and Populations. Plenum, New York.

—. 1979. Biosystematic studies in *Lycopersicon* and closely related species of *Solanum*. Pp. 667–677 *in* J. G. Hawkes, R. N. Lester & A. D. Skelding (editors), The Biology and Taxonomy of Solanaceae. Linn. Soc. Symp. Ser. 7. Academic Press, New York.

—. 1982a. The potential of exotic germplasm for tomato improvement. Pp. 1–28 *in* I. K. Vasil, W. R. Scowcroft & K. J. Frey (editors), Plant Improvement and Somatic Cell Genetics. Academic Press, New York.

—. 1982b. Genetic relationships between self-incompatibility and floral traits in the tomato species. Biol. Zentralbl. 101: 185–198.

—. 1986a. Germplasm resources in the wild tomato species. Acta Hort. 190: 39–47.

—. 1986b. Reproductive isolation in the *Lycopersicon peruvianum* complex. Pp. 477–495 *in* W. G. D'Arcy (editor), Solanaceae, Biology and Systematics. Columbia Univ. Press, New York.

—. 1987. Genetic resources in *Lycopersicon*. Pl. Biol. 4: 17–26.

—. 1988. Tomato-like nightshades: Affinities, autoecology, and breeders opportunities. Econ. Bot. 42: 145–154.

— & M. Holle. 1990. Andean *Lycopersicon esculentum* var. *cerasiforme*: Genetic variation and its evolutionary significance. Econ. Bot. 44: 69–78.

— & R. Lamm. 1955. Biosystematic studies on the status of *Lycopersicon chilense*. Amer. J. Bot. 42: 663–675.

— & S. D. Tanksley. 1981. Genetic variation in *Solanum pennellii*: Comparison with two other sympatric tomato species. Pl. Syst. Evol. 139: 11–45.

—, E. Kesicki, J. F. Fobes & M. Holle. 1976. Genetic and biosystematic studies on two new sibling species of *Lycopersicon* from Interandean Peru. Theor. Appl. Genet. 47: 55–68.

—, J. F. Fobes & M. Holle. 1977. Genetic variation in *Lycopersicon pimpinellifolium*: Evidence of evolutionary change in mating systems. Pl. Syst. Evol. 127: 139–170.

—, M. Holle & R. W. Thorp. 1978. Rates of cross-pollination in *Lycopersicon pimpinellifolium*: Impact of genetic variation in floral characters. Pl. Syst. Evol. 129: 31–44.

—, J. F. Fobes & S. D. Tanksley. 1979. Evolution of mating systems in *Lycopersicum hirsutum* as deduced from genetic variation in electrophoretic and morphological characters. Pl. Syst. Evol. 132: 279–298.

—, J. W. Deverna, R. T. Chetelat & M. A. Stevens. 1987. Potential contributions of wide crosses to improvement of processing tomatoes. Acta Hort. 200: 45–55.

—, H. Laterrot & J. Philouze. 1990. A revised key for the *Lycopersicon* species. Tomato Genet. Coop. Rep. 40: 31.

Rohlf, F. J. 1992. NTSYS-pc, Numerical Taxonomy and Multivariate System. Exeter, New York.

— & R. R. Sokal. 1981. Comparing numerical taxonomic studies. Syst. Zool. 30: 459–490.

SAS. 1995. JMP Software, Vers. 3.1. SAS Institute Inc., Cary, North Carolina.

—. 1998. SAS Proprietary Software, Vers. 7. SAS Institute Inc., Cary, North Carolina.

Sokal, R. R. 1986. Phenetic taxonomy: Theory and methods. Ann. Rev. Ecol. Syst. 17: 423–442.

Spooner, D. M., G. J. Anderson & R. K. Jansen. 1993. Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes, and pepinos (Solanaceae). Amer. J. Bot. 80: 676–688.

Stevens, M. A. & C. M. Rick. 1986. Genetics and breeding. Pp. 35–109 *in* J. G. Atherton & J. Rudich (editors), The Tomato Crop: A Scientific Basis for Improvement. Chapman and Hall, London.

Swofford, D. L. 1998. PAUP*: Phylogenetic Analysis Using Parsimony, Vers. 4d64. Sinauer, Sunderland, Massachusetts.

Tanksley, S. D. & F. Loaiza-Figueroa. 1985. Gametophytic self incompatibility is controlled by a single locus on chromosome 1 in *Lycopersicon peruvianum*. Proc. Natl. Acad. Sci. U.S.A. 82: 5093–5096.

— & S. R. McCouch. 1997. Seed banks and molecular maps: Unlocking genetic potential from the wild. Science 277: 1063–1066.

Taylor, I. B. 1986. Biosystematics of the tomato. Pp. 1–34 *in* J. G. Atherton & J. Rudich (editors), The Tomato Crop: A Scientific Basis for Improvement. Chapman and Hall, London.

Villagran, C., M. T. K. Arroyo & C. Marticorena. 1983. Efectos de la desertización en la distribución de la flora andina de Chile. Revista Chilena Hist. Nat. 56: 137–157.