Pl Syst Evol 268: 159–175 (2007)
DOI 10.1007/s00606-007-0567-5
Printed in The Netherlands

Plant Systematics and Evolution

The discovery and phylogenetic implications of a novel 41 bp plastid DNA deletion in wild potatoes

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Received 26 December 2006; Accepted 9 May 2007; Published online 1 August 2007

Abstract. Insertions and deletions (indels) are common in intergenic spacer regions of plastid DNA and can provide important phylogenetic characters for closely related species. For example, a 241-bp plastid DNA deletion in the trnV-UAC/ndhC intergenic spacer region has been shown to have major phylogenetic importance in determining the origin of the cultivated potato. As part of a phylogenetic study of the wild potato *Solanum* series *Piurana* group we screened 199 accessions of 38 wild potato species in nine of the 19 tuber-bearing (*Solanum* section *Petota*) series that have not been examined before for indels in the *trn*V-UAC/*ndh*C intergenic spacer region. A novel 41 bp deletion (but no 241 bp deletion) was discovered for 30 accessions of three species: *S. chiquidenum* (5 of 10 accessions), *S. chomatophilum* (19 of 28), and *S. jalcae* (6 of 6). Accessions with and without this deletion are found throughout much of the north-south range of all three species in northern and central Peru, but not east of the Marañón River. Multivariate morphological analyses of these 44 accessions showed no morphological associations to the deletion. The results suggest extensive interspecific gene flow among these three species, or a common evolutionary history among species that have never been suggested to be interrelated.

Keywords: Plastid DNA; Potato; *Solanum* sect. *Petota*; *trn*V-UAC/*ndh*C intergenic spacer

Introduction

Plastid genomes of land plants are highly conserved in both gene order and gene content. Currently, there are 47 completely sequenced plastid genomes from various plant lineages (Raubeson and Jansen 2005), 23 of these from crop plants (Daniell et al. 2006). Plastid characters can provide phylogenetically useful information at various taxonomic levels (Palmer et al. 1988, Daniell et al. 2006). Comparison of the genomes of four species in the Solanaceae has shown the phylogenetic utility of insertions and deletions in eight intergenic spacer

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regions. This paper concentrates on the survey of one highly analyzed plastid DNA intergenic spacer region, *trn*V-UAC/*ndh*C in wild potatoes, *Solanum* L. section *Petota* Dumort.

There are about 190 wild potato species (Spooner and Salas 2006), growing in a variety of habitats, and distributed from the southwestern United States to Argentina, Chile, and Uruguay (Hijmans and Spooner 2001). They are morphologically very diverse, and different taxonomic interpretations of this group are common. Due to the large size of the group we are conducting focused systematic studies throughout putatively related and taxonomically difficult species.

This study focuses on species in *Solanum* series *Piurana* Hawkes and additional species from other series that we think are related to this series. Hawkes (1990) included 15 species in series *Piurana*, distributed from southern Ecuador to central Peru. The limits of the series and its constituent species are unresolved. The most distinctive morphological characters of series *Piurana* are: 1) the globose to ovoid fruits, 2) coriaceous and glossy leaves (Correll 1962, Hawkes 1990, Ochoa 1999), and 3) moniliform tubers, arranged like beads along the stolons, unlike the more typical arrangement of single tubers placed at stolon ends (Spooner and Salas 2006).

Based on plastid DNA restriction site data, Spooner and Castillo (1997) supported four clades in section *Petota*, not the many series of other authors. One of these clades contained all described species in series *Piurana*, and species from other series (Fig. 1). Castillo and Spooner (1997) added yet other species to this clade. Because of uncertainties about the limits of the series, we are investigating many species that may be related to series *Piurana*, based on insights from these plastid DNA restriction site phylogenetic studies and hypotheses of relationships based on associations of the above three morphological characters (Spooner and Salas 2006). These include all species of series *Piurana*, and some species in series *Conicibaccata*, *Cuneoalata*, *Ingifolia*, *Megistacroloba*, *Olmosiana*, *Simplicissima*, *Tuberosa*, and *Yungasensa*.

Hosaka (2002, 2003) found a 241-bp deletion (Kawagoe and Kihuta 1991) in the trnV-UAC/ndhC intergenic spacer region that characterized many accessions of the wild potato species *S. berthaultii*, *S. tarijense*, *S. neorossii*, and most landrace populations of *S. tuberosum* from Chile, but not any other wild species. This deletion has been instrumental in discovering the origins of the cultivated potato in Europe (Hosaka 2002, 2003; Spooner et al. 2005; Rios et al. in press). This study reports our discovery of another taxonomically significant plastid deletion in the *trn*V-UAC/*ndh*C intergenic spacer region.

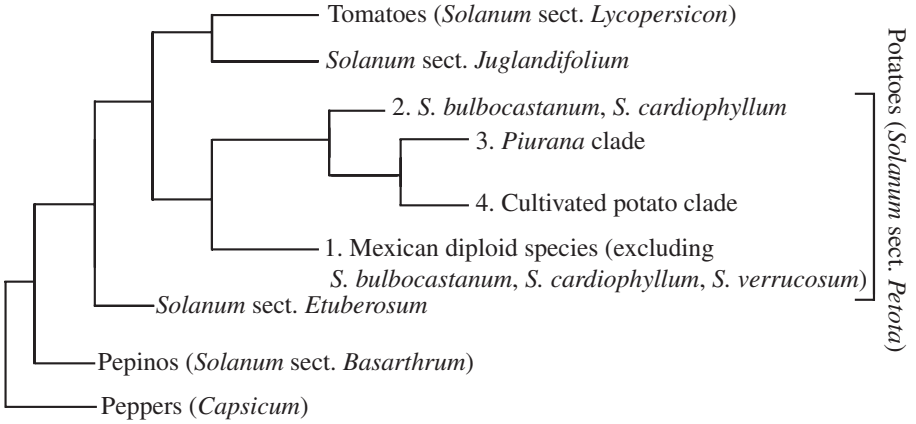

Fig. 1. Cladistic relationships of potato, tomato, and outgroups, sensu Spooner and Castillo (1997) and Castillo and Spooner (1997) as described in text, showing the four clades in potato

Table 1. The 199 accessions of 38 wild potato species of *Solanum* section *Petota* examined for deletions in the intergenic region flanking the 3' end of the *trn*V-UAC gene, and the two accessions of *S. tuberosum* (Andigenum Group from Dept. Amazonas, Peru; Chilotanum Group from the Chonos Archipelago Chile), used for comparative analyses. The last column designates three states in this region: "A" is "wild type" with no deletion, "P" possesses a 41 bp deletion, and "T" (present only in *S. tuberosum* Chilotanum Group) possesses a 241 bp deletion. See Fig. 3 for positions of these deletions

Species and series affiliations (Hawkes, 1990)	Collector number[1]	Genebank number[2]	plastid DNA deletion
Series *Conicibaccata*			
Solanum chomatophilum Bitter	COR P862	266387	P
	HAW 2417	762649	P
	HAW 2433	310990	P
	OCH 11061	761173	A
	OCH 11755	761271	P
	OCH 13199	761541	P
	OCH 13204	761546	P
	OCH 13208	761549	P
	OCH 13212	761551	A
	OCH 13288	761577	P
	OCH 13325	761582	A
	OCH 13367a	763277	P
	OCH 1512a	763188	P
	OCH 15302	568979	A
	OCH 1664	760057	A
	OCHS 12561	762569	A
	OCHS 12570	762575	P
	OCHS 14485	762054	P
	OCHS 14486	762055	P
	OCHS 16060	763609	P
	OCH S-42	760911	A
	OCH S-71	760913	A
	SSTS 7322	762941	P
	SSTS 7323	762942	P
	SSTS 7327	762946	P
	SSTS 7341	762960	A
	SSTS 7347	762966	A
	UGN 5416	310943	P
S. contumazaense Ochoa	310988x365329	365329	A
	OCH 14751	762118	A
S. paucijugum Bitter	OCH 13371	761595	A
	OCH 13377	761597	A
	SCL 5071	762798	A
	SCL 5084	762800	A
	SCLG 5151	762816	A
	SCLp 5096a	583303	A
	SLE 5094	762802	A
	SLE 5096b	762803	A
	SLE 5129	762810	A
	SLT 5130	763806	A

Table 1. (Continued)

Species and series affiliations (Hawkes, 1990)	Collector number[1]	Genebank number[2]	plastid DNA deletion
Series *Cuneoalata* Hawkes			
S. anamatophilum Ochoa	SSTH 7391	762909	A
S. infundibuliforme Phil.	OKA 4550	472887	A
	OKA 4557	472888	A
S. peloquinianum Ochoa	OCH 13231	761555	A
	SSTS 7336	762955	A
	SSTS 7337	762956	A
Series *Ingifolia* Ochoa			
S. ingifolium Ochoa	OCHS 11614	761235	A
S. raquialatum Ochoa	OCH 13947	761863	A
	OCH 13950	761864	A
Series *Megistacroloba* Cárdenas and Hawkes			
S. sogarandinum Ochoa	OCH 13006	761465	A
	OCH 13013a	761471	A
	OCH 1440	762987	A
	OCH S-54	760018	A
	SSTS 7332	762951	A
Series *Olmosiana* Ochoa			
S. olmosense Ochoa	OCH 13348	761589	A
Series *Piurana* Hawkes			
S. acroglossum Juz.	OCHS 11297	761070	A
S. albornozii Correll	OCHS 11007	761164	A
	SCLp 5030	561635	A
	SCLp 5032	561636	A
	SCLp 5033	561637	A
S. cantense Ochoa	OCHL 14828	762130	A
	SSTH 7368	762886	A
	SSTH 7370	762888	A
	SSTH 7372	762890	A
	SSTH 7382	762900	A
S. chilliasense Ochoa	OCH 13350	761590	A
	SCLp 5057	567821	A
S. hypacrarthrum Bitter	OCH 11607	473477	A
	OCH 11692	761259	A
	OCHS 11308	761204	A
	OCHS 14715	762104	A
	OCHS 14731	762111	A
S. jalcae Ochoa	OCHS 16021	763601	P
	OCHS 16036a	763602	P
	OCHS 16060a	763610	P
	SSTS 7316	762935	P
	SSTS 7317	762936	P
	SSTS 7320	762939	P
	SSTS 7325	762944	P
S. pascoense Ochoa	OCHS 11858	761305	A

Table 1. (Continued)

Species and series affiliations (Hawkes, 1990)	Collector number[1]	Genebank number[2]	plastid DNA deletion
S. paucissectum Ochoa	HUA 974	760956	A
	OCH 11628	761241	A
	OCH 11633	761246	A
	OCH 11634	761247	A
	OCHS 14816	762124	A
	OCHS 14817	762125	A
	OCHS 14818	762126	A
S. piurae Bitter	365365x473501	365365	A
	HUA 964	760953	A
	OCH 13959	761868	A
	OCHS 11615	761072	A
S. solisii Hawkes	OCHS 10990	761161	A
S. tuquerrense Hawkes	CCC 5126	498177	A
	HAW 2547	762711	A
	HOHL 298	546033	A
	OCH 13835	761834	A
	SCL 5098	762804	A
	SCL 5100	762805	A
	SCL 5118	763764	A
	SCLp 5007	561628	A
	SCLp 5022	561631	A
	SCLp 5097	561645	A
	SCLp 5111	561646	A
	SL 5023	762788	A
S. x blanco-galdosii Ochoa	OCH 10673	762524	P
	OCH 13009	761466	A
	OCH 5169	442702	A
	SSTS 7339	762958	A
Series *Simplicissima* Ochoa			
S. simplicissimum Ochoa	OCHS 15147	762233	A
	SSTH 7378	762896	A
Series *Tuberosa* (Rydb.) Hawkes			
S. andreanum Baker	CCC 5142	762677	A
	CCC 5186	597668	A
	COR CO491	247360	A
	CPLS 1226	567813	A
	HAW 2546	320345	A
	SCL 5101	762806	A
	SCLG 5153	762817	A
	SCLG 5155	762818	A
	SCLp 5126	561648	A
	SCLp 5133	561658	A
	SCLp 5155	561661	A
S. augustii Ochoa	OCHS 12596	762631	A
	OCHS 12602	762633	A

Table 1. (Continued)

Species and series affiliations (Hawkes, 1990)	Collector number[1]	Genebank number[2]	plastid DNA deletion
S. cajamarquense Ochoa	OCHS 16063	762608	A
	OCHS 16118	762616	A
	OCHS 16119	762617	A
	OCHS 16121	762619	A
	OCHS 16122	762620	A
	OCHS 16123	762621	A
	OCHS 16124	762622	A
	OCHS 16241	763011	A
S. chancayense Ochoa	EBS 2807	338615	A
	OCH 11250	761180	A
	OCHS 11250	761180	A
S. chiquidenum Ochoa	OCH 13963	761870	A
	OCHS 11059	761069	P
	OCHS 12543	762553	P
	OCHS 12566	762573	A
	OCHS 12568	762574	A
	OCHS 14482	762052	P
	OCHS 14487	762056	P
	OCHS 15240	762263	A
	SSTS 7321	762940	A
	SSTS 7331	762950	P
S. dolichocremastrum Bitter	OCH 12071	761043	A
	OCH 12072	761437	A
	OCH 12074	761439	A
	OCH 13013	761470	A
	OCHS 16202	763618	A
	OCHS 16205	763621	A
	OCHS 16207	763623	A
	OCHS 16208	763624	A
	OCHS 16209	762998	A
	OCHS 16218	763632	A
	SSTS 7351	762970	A
	SSTS 7353	762972	A
S. guzmanguense Whalen and Sagást.	OCHS 15989	762634	A
	OCHS 15991	762636	A
S. huarochiriense Ochoa	OCH 11325	761215	A
	OCH 11331	761220	A
	OCH 11334	761223	A
	OCH 11335	761224	A
	OCH 11699	761265	A
	SSTH 7388	762906	A
	SSTS 7311	762930	A
S. humectophilum Ochoa	OCH 11753	761052	A
S. immite Dunal	365330x365331	365331	A
	OCH 11689	761040	A
	OCHS 14491	762059	A
	SSTS 7315	762934	A

Table 1. (Continued)

Species and series affiliations (Hawkes, 1990)	Collector number[1]	Genebank number[2]	plastid DNA deletion
S. marinasense Vargas	OCH 13608	761669	A
	OCH 13620	761678	A
	OCH 13673	761714	A
	OCH 13689	761731	A
	OCH 13738	761775	A
	OCH 13748	761783	A
	OCH 14384	762024	A
	OCH 14386	762026	A
	OCHB 15687	762390	A
	OCHRO 14417	762051	A
	SS 7209	762828	A
S. mochiquense Ochoa	EBS 2764	338616	A
	GLKS 127/1	498411	A
	OCH 10728a	762989	A
	OCH 1822	760103	A
	OCHS 14870	761037	A
	OCHS 15993	762637	A
	OCHS 16232	763002	A
	OCH S-20	760009	A
S. multiinterruptum Bitter	OCH 12066	761432	A
	OCH S-27	762995	A
	OCH S-36	760859	A
S. scabrifolium Ochoa	OCH S-60	760020	A
S. tuberosum Andigenum Group		704429	A
S. tuberosum Chilotanum Group		703611	T
Series *Yungasensa* Correll			
S. huancabambense Ochoa	GLKS 155/1	498413	A
	HUA 966	458400	A
	OCH 11619	761238	A
	OCH 11626	761239	A
	OCH 11627	761240	A
	OCHS 14815	762123	A

[1] Collector codes are: CCC, Colección Central Colombiana; COR, D. Correll; CPLS, Castillo et al.; EBS, H. Ross; GLKS, Institute of Plant Breeding, Gross-Lusewitz, Germany; HAW, J. G. Hawkes; HOHL, Hoopes et al.; HUA, Z. Huamán; OCH, C. Ochoa; OCHB, C. Ochoa and O. Blanco; OCHL, C. Ochoa and P. López Camarena; OCHRO, C. Ochoa and R. Ortega; OCHS, C. Ochoa and A. Salas; OKA, K. A. Okada; SCL, SCLG, SCLp, SLE, SLT, SSTH, SSTS, Spooner et al.; SL, D. Spooner and L. López; SS, D. Spooner and A. Salas; UGN, D. Ugent

[2] The 700,000 genebank series numbers are from the International Potato Center; the 500,000 series numbers and below are from the United States Potato Genebank

Materials and methods

Accessions examined and plastid DNA, extraction PCR and sequencing protocols.

Total genomic DNA was isolated from young leaves of single plants of 199 accessions belonging to 38 species from nine taxonomic series of Hawkes (1990) (Table 1), using standard protocols adapted at CIP (Ghislain et al. 1999). DNA quantity were estimated by comparison with the upper band (11,490 bp) of $l\mu$g of Lambda DNA (Gibco-BRL, Gaithersburg, Md.) digested with *Pst*I and

subjected to electrophoresis on a 1% agarose gel ethidium bromide-stained.

A pair of primers in the *trn*V-UAC/*ndh*C intergenic spacer region (5′AAG TTT ACT CAC GGC AAT CG 3′ and 5′GGA GGG GTT TTT CTT GGT TG 3′) was used based on Hosaka (2002). PCR reactions were performed in a 10μl volume containing 100 mM Tris-HCl, (Promega Corp. Madison WI), 2.5 mM $MgCl_2$ (Promega), 0.25 mM of each dNTP (Promega), 0.5μM of each primer (forward and reverse, UW- Biotechnology Center), 0.25 units of Taq polymerase (Promega) and 10 ng of genomic DNA. PCR was carried out in a BIO-RAD MyCycler thermal cycler (Bio-Rad), set to the following program: 1 cycle of 3 min at 94°C, 40 cycles of 30 sec at 94°C, 30 sec at 55°C, 1 min at 72°C and one final cycle at 72°C for 5 min. The amplification products were run on a 3% agarose gel and stained with ethidium bromide and viewed with ultraviolet light. We ran these with representatives of both the Andigenum and Chilotanum Groups (Table 1).

For DNA sequencing of this region, amplified plastid DNA bands from sixteen accessions were cut and purified from the gel using the Zymoclean^{TM} Gel DNA Recovery Kit. They were cloned into a pGEM-T easy vector system (Promega Corp. Madison - WI). We cloned this region to insure that we recovered the entire length of the originally published wild type plastid region without the 241-bp deleted fragment for comparison with our new results. Plasmid DNA was purified from transformed *E. coli* JM109 using the Wizard Plus SV Minipreps DNA purification System (Promega Corp. Madison, WI). Sequencing reactions consisted of 1μl of BigDye Terminator v. 3.1 mix (Applied Biosystems), 1.5μl of dilution buffer (Applied Biosystems), 5–20 pmol of primer, and 0.2μg of template DNA in a final reaction volume of 10μl. The sequencing reaction was carried out in a BIO-RAD MyCycler thermal cycler (Bio-Rad), set to the following program: 1 cycle of 3 min at 95°C, 40 cycles of 25 sec at 96°C, 20 sec at 50°C, 5 min at 60°C and one final cycle at 72°C for 7 min. Excess dye terminators were removed using CleanSeq magnetic bead sequencing reaction clean up kit from Agencourt Biosciences. The samples were resuspended off of the beads in 50μl of ddH_2O. 20μl of each sample was loaded into a 96 well PCR plate, and the plate loaded onto the sequencers according to the manufacturer's instructions. Samples were electrophoresed on an Applied Biosystems 3730xl automated DNA sequencing instrument, using 50 cm capillary arrays and POP-7 polymer. Data were analyzed using PE-Biosystems version 3.7 of Sequencing Analysis. Five clones were sequenced for each accession. DNA sequences were edited using Staden package V.1.6.0. (Staden 1996), and sequence alignments were conducted using Clustal X (Thompson et al. 1997). The sequences obtained for the accession of *S. tuberosum* Andigenum group was used for alignment because of the lack of deletion.

Morphological evaluations. Based on results of the plastid DNA analysis (below) we conducted morphological morphometric analyses of ten accessions of *S. chiquidenum*, 28 of *S. chomatophilum*, and six of *S. jalcae*. All the evaluations were conducted on plants grown in pots in greenhouses at the International Potato Center (CIP) Research Station in the central Peruvian Andes of Huancayo, Peru (elevation 3200 m above sea level, 12°8' S, 75°8' W). A list of the evaluated characters is found in Table 2. Two repetitions were planted in separate greenhouses. Five plants were grown for each accession in pots in each greenhouse, and measurements were taken from three plants for each accession in each greenhouse. Hand pollination was performed to aid fruit formation. Leaf measurements were taken from the middle leaf of each plant just before or during flowering. Fruits were measured when fully mature and tubers were evaluated at senescence. Colors were assessed for corollas and tubers using the RHS Colour Chart (Royal Horticultural Society 2001). A Hunter Lab Color Quest 45/0 colorimeter was used to obtain CIELab values from the color charts. The CIELab values are a color model used conventionally to describe all the colors visible to the human eye. The L* parameter represents the lightness of the color or luminance (L* = 0 (black) and L* = 100 (white)), a* the position between green (when negative) and magenta (when positive), and b* the position between yellow (negative values) and blue (positive values). Since the L*a*b* values showed a high correlation between them, only one of them was selected according to its 'coverage' for the tuber skin, tuber flesh, corolla lobe and corolla ray colors (abaxial and adaxial). For tuber skin color the b* parameter was chosen because the tubers

Table 2. Characters used in the morphological analysis of *Solanum chiquidenum*, *S. chomatophilum*, and *S. jalcae*. See Materials and Methods for method for measuring colors unless as stated below

Stem characters

1. Diameter of stem at the middle part of the plant (cm). 2. Stem color (1) green, (2) green mottled with purple, (3) purple. 3. Stem morphology (1) circular, (2) polygonal, (3) triangular. 4. Width of stem wings (cm). 5. Plant height (cm).

Leaf characters (from leaves taken at the middle of flowering plants)

6. Length of leaf (cm). 7. Length of terminal leaflet lamina (cm). 8. Length of petiolule of terminal leaflet (cm). 9. Number of pairs of lateral leaflets. 10. Number of primary interstitial leaflets. 11. Number of secondary interstitial leaflets. 12. Margin of leaflets (1) straight, (2) undulate, (3) sinuate. 13. Width of terminal leaflet 5 mm from apex (cm). 14. Base shape of terminal leaflet: (1) equilateral, (2) attenuate, (3) auriculate, (4) cordate, (5) cuneate, (6) hastate, (7) oblique, (8) rounded, (9) sagittate, (10) truncate. 15. Number of interstitial leaflets at base of terminal leaflet. 16. Length of petiolule of the most distal lateral leaflet (cm). 17. Length of the largest interstitial leaflet (cm). 18. Length of widest point of the most distal lateral leaflet (cm). 19. Length of the most distal lateral leaflet (cm). 20. Width of most distal lateral leaflet 5 mm from apex (cm). 21. Width of decurrent tissue under the most distal lateral leaflet (cm) measured 0.5 cm below the insertion point of leaf in the rachis. 22. Width of secondary lateral leaflet between apexes (cm). 23. Length between apices of third most distal lateral leaflets (cm). 24. Color of abaxial surface of leaf (1) light green, (2) medium green, (3) dark green, (4) purple green. 25. Color of abaxial surface of leaf (1) green, (2) green with purple veins, (3) green with purple spots, (4) completely purple. 26. Density of pubescence adaxial (number of hairs/cm^2). 27. Length of pubescence on adaxial surface of leaf (mm). 28. Density of pubescence on adaxial surface of leaf (number of hairs/cm^2). 29. Length of pubescence on abaxial surface of leaf (mm). 30. Ratio: leaf length/width of leaf. 31. Ratio: length of axis of widest point of leaf to apex/length of leaf. 32. Ratio: length of terminal leaflet lamina/width of terminal leaflet. 33. Ratio: length of axis of widest point of terminal leaflet to apex/length of terminal leaflet lamina. 34. Ratio: length of most distal lateral leaflet/width of most distal lateral leaflet. 35. Ratio: length from axis of widest point of most distal lateral leaflet to apex/length of most distal lateral leaflet.

Floral characters

36. Density of calyx pubescence (hairs cm^2). 37. Length of calyx pubescence (mm). 38. Length of the peduncle (cm). 39. Length of pedicel (cm). 40. Length of pedicel from its base to articulation (cm). 41. Ratio: length of pedicel articulation/length of pedicel. 42. Number of peduncle forks. 43. Number of flowers per inflorescence. 44. Length of calyx acumen (cm). 45. Length of calyx lobe (cm). 46. Ratio: length of calyx lobe/width of calyx lobe. 47. Radius of corolla (cm). 48. Ratio: Length from center of corolla to base of corolla lobes/radius of corolla. 49. Width of corolla lobe at base of junction of corolla lobes (cm). 50. Ratio: Width of corolla lobe at base of junction of corolla lobes/length from base to tip of corolla lobe. 51. Length of anther (cm). 52. Length of style exertion from apex of anthers to apex of stigma (cm). 53. Shape of stigma (1) capitate, (2) clavate and (3) lobate. 54. Ratio: diameter of style/diameter of stigma. 55. Diameter of stigma (mm). 56. Length of stigma (mm). 57. Ratio: diameter of stigma/length of stigma. 58. Polymorphism in the size of the anthers, (1) no polymorphism, (2) two different sizes, (3) three different sizes, (4) four different sizes, (5) all different 59. Purple color in rachis at leaflets insertion point (1) present (2) absent. 60. Color of abaxial corolla interpetolar tissue. 61. Color of adaxial corolla rays. 62. Color of abaxial corolla interpetolar tissue. 63. Color of abaxial corolla rays.

Tuber characters

64. Tuber type (1) moniliform, (3) at the end of the stolon, (5) swellings along the stolon. 65. Tuber length $\times$ width. 66. Tuber length (cm). 67. Number of eyes on tuber. 68. Ratio: tuber weight per plant (gr)/number of tubers. 69. Number of tubers. 70. Tuber primary color of external surface. 71. Tuber flesh color. 72. Tuber eye color. 73. Tuber secondary color of external surface.

Table 2. (Continued)

Fruit characters
74. Length of fruit (cm). 75. Width of fruit at 0.5 cm above the fruit apex (cm). 76. Ratio: length of fruit/width at widest point of the fruit. 77. Ratio: length of fruit/width at its narrowest point. 78. Ratio: width of fruit at its widest point/width of fruit at 0.5 cm above the fruit apex. 79. Ratio: width of fruit at its widest point/width of fruit at its narrowest point. 80. Purple dot in the fresh mature seeds (1) present, (2) absent. 81. Fruit color (1) uniform, (2) mottled. 82. Texture of the external surface of the fruit (1) rugose, (2) smooth.

scored a yellowish coloration while L* and a* were not included for tuber color characters. The same criteria was used for the corolla colors (lobes and rays) where the parameter a* was selected.

Principal components analysis (PCA) of averages from each measurement from both replicates was performed using NTSYS 2.02K program (Applied Biosystematics, Setauket, NY, USA). The geographical distribution of the 44 accessions of *S. chiquidenum*, *S. chomatophilum*, and *S. jalcae* were mapped using DIVA-GIS v5.2 (Hijmans et al. 2001).

Results

Plastid DNA deletion. Our PCR screening found a relatively small deletion (clearly shorter than the well-characterized 241-bp deletion) in the trnV-UAC/ndhC intergenic spacer region in 5 of 10 accessions of *S. chiquidenum*, 19 of 28 accessions of *S. chomatophilum*, and all six accessions of *S. jalcae*.

We obtained sequences of representative accessions both possessing and lacking the deletion for 14 accessions of these three species, covering their geographical range (Table 3, Figs. 2, 3). The five accessions of *S. chiquidenum* or *S. chomatophilum* lacking the deletion were identical to *S. tuberosum* Andigenum Group except for very minor base pair changes, but no indel differences (Fig. 3). The nine accessions of *S. chiquidenum*, *S. chomatophilum*, and *S. jalcae* that had the deletion showed an identical deletion size (41 bp) and position, somewhat midway between the 241 bp deletion; specifically 118 bp from the 5′ beginning and 82 bp from its end (Fig. 3).

Morphology. Morphological analyses grouped all 34 accessions *S. chomatophilum* and *S. jalcae*, separate from the ten accessions of *S. chiquidenum*. There was no clustering of plastid deletion variants within any of the species (Fig. 4).

Geography. We mapped all 44 accessions of *S. chiquidenum*, *S. chomatophilum*, and *S. jalcae*. The examined accessions represent nearly the entire geographic ranges of these species (Ochoa 1999), except for accessions of *S. chomatophilum* not sampled from the Department of Lima in the southern part of the range (Fig. 2). Although accessions with its deletion span the north-south range of these species as a group, accessions lacking the deletion are absent east of the Marañón River.

Discussion

We were surprised at the results showing *S. chiquidenum* sharing the 41 bp deletion with *S. chomatophilum* and *S. jalcae*, because it is so morphologically different from these two species. Our morphological analyses showed the latter two to be so similar as to possibly be conspecific. This matched our intuitive observations as we had difficulty distinguishing *S. chomatophilum* and *S. jalcae* in the field (Salas et al. 2001) and when measuring these plants in our greenhouse study. Taxonomic treatments of Correll (1962), Hawkes (1990), and Ochoa (1999), however, did not see the similarities as they placed both *S. chomatophilum* and *S. jalcae* in different series (Table 4). Spooner and Salas (2006) suggested that all three species were in series *Piurana* but did not speculate about their interrelationships within this series.

Daniell et al. (2006) compared the plastid DNA intergenic spacers greater than 11 bp of

Table 3. Collector number and locality data of the 44 accessions of *Solanum chiquidenum*, *S. chomatophilum*, and *S. jalcae* surveyed for the 41 bp deletion in the intergenic region flanking the 3′ end of the trnV-UAC gene. All accessions are mapped in Fig. 2. The deletion codes A and P follow Table 1; an asterisk indicates the 14 accessions sequenced in this region as shown in Fig. 3

Species, collector number[1]	Locality	plastid DNA deletion
Solanum chiquidenum Ochoa		
OCH 13963	PERU: Cajamarca, Contumazá, Cajon.	A
OCHS 11059	PERU: Cajamarca, Contumazá, Cerro Chungarran.	P*
OCHS 12543	PERU: Cajamarca, Cajabamba, San Nicolas.	P
OCHS 12566	PERU: Cajamarca, Hualgayoc, Colquerumi.	A*
OCHS 12568	PERU: Cajamarca, Hualgayoc.	A
OCHS 14482	PERU: La Libertad, Otuzco, Cerro Songo.	P*
OCHS 14487	PERU: La Libertad, Otuzco, Cerro Songo.	P
OCHS 15240	PERU: Cajamarca, Cutervo, La Ramada.	A
SSTS 7321	PERU: La Libertad, Santiago de Chucon, Montana La Botica.	A
SSTS 7331	PERU: La Libertad, Julcan, Chorro Blanco, 1 km E of community of Victor Julio Roselle, SW of Corrapaldy Chico.	P*
S. chomatophilum Bitter		
COR P862	PERU: Cajamarca near the entrance to Hacienda Porcon.	P
HAW 2417	PERU: Ancash, Pallasca. Near Consuzo, Cerro Huaura, minas de Huaura, San Luis.	P
HAW 2433	PERU: Cajamarca, Hacienda Porcon.	P
OCH 11061	PERU: Amazonas, Luya Cutra Cuello.	A*
OCH 11755	PERU: La Libertad, Huamachuco Pampa de Condor.	P
OCH 13199	PERU: Cajamarca, Hualgayoc Quinuapampa.	P
OCH 13204	PERU: Cajamarca, Chota, Cerro Calvario.	P
OCH 13208	PERU: Cajamarca, Celendin, Tablacucho.	P*
OCH 13212	PERU: Cajamarca, Cajamarca, Silleropata.	A
OCH 13288	PERU: La Libertad, Sanchez Carrion, Aricapampa.	P*
OCH 13325	PERU: La Libertad, Pataz, between Gachil and Urpay.	A*
OCH 13367a	PERU: Pasco	P
OCH 1512a	PERU: Pasco	P*
OCH 1664	PERU: Amazonas, Chachapoyas, Cerro Tinaja.	A
OCHS 12561	PERU: Cajamarca, Celendin, Piedra Grande.	A*
OCHS 12570	PERU: Cajamarca, Hualgayoc, Mina Carolina.	P
OCHS 14485	PERU: La Libertad, P. Otuzco, Cerro Songo.	P
OCHS 14486	PERU: La Libertad, P. Otuzco, Cerro Songo	P
OCHS 16060	PERU: Cajamarca, Cajamarca, Sinsi.	P
OCH S-42	PERU: Amazonas, Cerro Canal, south of Cerro Campanario.	A
OCH S-71	PERU: Amazonas, Pomacocha, between Leimebamba and Calla-Calla.	A
SSTS 7322	PERU: La Libertad, Santiago de Chuco, Laguna de Torro, 11 km E and N of Quiruvilca on the road to Huamachuco.	P
SSTS 7323	PERU: La Libertad, Santiago de Chuco, Laguna de Torro, on N side of road, 11 km E and N of Quiruvilca on the road to Huamachuco.	P
SSTS 7325	PERU: La Libertad, Sanchez Carrion, Casbambul, on the road to Huamachuco.	P

Table 3. (Continued)

Species, collector number[1]	Locality	plastid DNA deletion
SSTS 7327	PERU: La Libertad, Sanchez Carrion, Macullada, 14 km S of Molina Viejo, on the road to Huamachuco to Pataz.	P*
SSTS 7341	PERU: La Libertad, Pataz, Punya, 30 km N of Huacaspata.	A*
SSTS 7347	PERU: Ancash, Yungay, Yurac Corral (Huischa). 18.8 km E of park entrance into Parque Nacional Llanganuco, 11.0 km NE of NE end of the 2nd part of Laguna Llanganuco on the rod to Piscobamba.	A
UGN 5416	PERU: Cajamarca, Cajamarca, Hacienda Porcon.	P
S. jalcae Ochoa		
OCHS 16021	PERU: Cajamarca, Cajamarca, China Linda.	P*
OCHS 16036a	PERU.	P
OCHS 16060a	PERU: Cajamarca, Cajamarca.	P
SSTS 7316	PERU: La Libertad, Otuzco, 1 km W of town of Sanro (below Mt. Sanro), in between Motil and Shorey, 200–300 m S of road.	P
SSTS 7317	PERU: La Libertad, Santiago de Chuco, 9.8 km E of town of Sanro (below Mt. Sanro), in between Motil and Shorey, 200–300 m N of road.	P*
SSTS 7320	PERU: La Libertad, Santiago de Chuco. 22.5 km from the police station in Shorey on the road to Santiago de Chuco.	P

[1] See Table 2 for collector codes

four Solanaceae genomes (*Atropa*, tobacco, tomato, potato). They found that only four of them had 100% sequence identity among all four genomes, but 21 regions had 100% sequence identity for potato and tomato. They reported that there are several insertions and deletions in the intergenic spacer regions for eight spacers, but none were reported for the region we examine for the *ndh*C-*trn*V. They report that in general, intergenic spacer regions are more variable and provide phylogenetically informative characters among closely related species. In agreement with Daniell et al. (2006), Kahlau et al. (2006) compared insertions and deletions between tomato and tobacco and the majority of insertions and deletions were located in noncoding spacer regions.

Our study builds on comparative data within section *Petota* that addresses the phylogenetic utility of plastid intergenic insertions and deletions. Our deletion survey adds to the work of Hosaka (2002) who surveyed 35 species we did not survey, distributed in four taxonomic series (*Yungasensa*, 3 species; *Megistacroloba*, 5; *Maglia* 1; *Tuberosa* 26 species). We add new observations for the series *Conicibaccata*, *Ingifolia*, *Olmosiana*, *Piurana*, and *Simplicissima*.

Prior to our study, two insertions and deletions were known from the *ndh*C-*trn*V intergenic region. 1) A 241 bp deletion in *S. tarijense* (found in 10 out of 29 examined accessions), *S. berthaultii* (4 of 32) and *S. neorossii* (2 out of 3), and in most accessions *S. tuberosum* Chilotanum Group cultivars from Chile and in modern advanced cultivars grown worldwide (Hosaka 2002). These results, and additional plastid DNA restriction site data, were used to suggest *S. tarijense* as a maternal contributor to the Chilean cultivars. Relative to the wild species, this 241 bp deletion is widely distributed throughout the range of *S. berthaultii*, *S. neorossii*, and *S. tarijense* from central

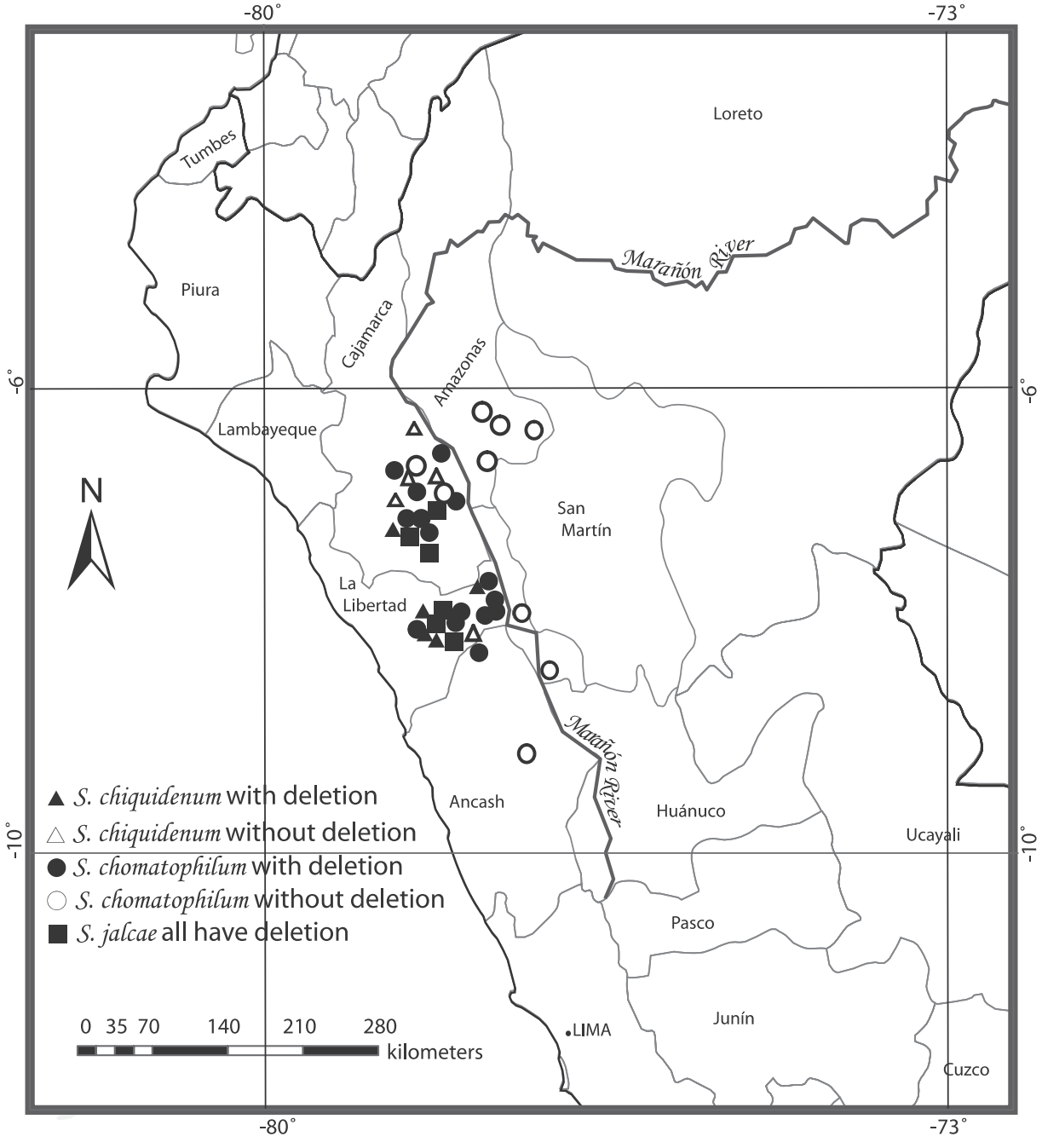

Fig. 2. Distribution of the examined accessions of *Solanum chiquidenum*, *S. chomatophilum*, and *S. jalcae* in central Peru, with indication of presence (solid symbols) or absence (open symbols) of the 41 bp deletion in the *trn*V-UAC/*ndh*C intergenic spacer region

Bolivia in Cochabamba Province, south to northern Argentina in Salta Province. 2) A 65 bp duplication was discovered in *ndh*C-*trn*V intergenic region in 3 of 29 accessions of *S. vernei* from Argentina. All three of these accessions are clustered in a small subset of the range of *S. vernei* in Tucumán Province and immediately adjacent Catamarca Province.

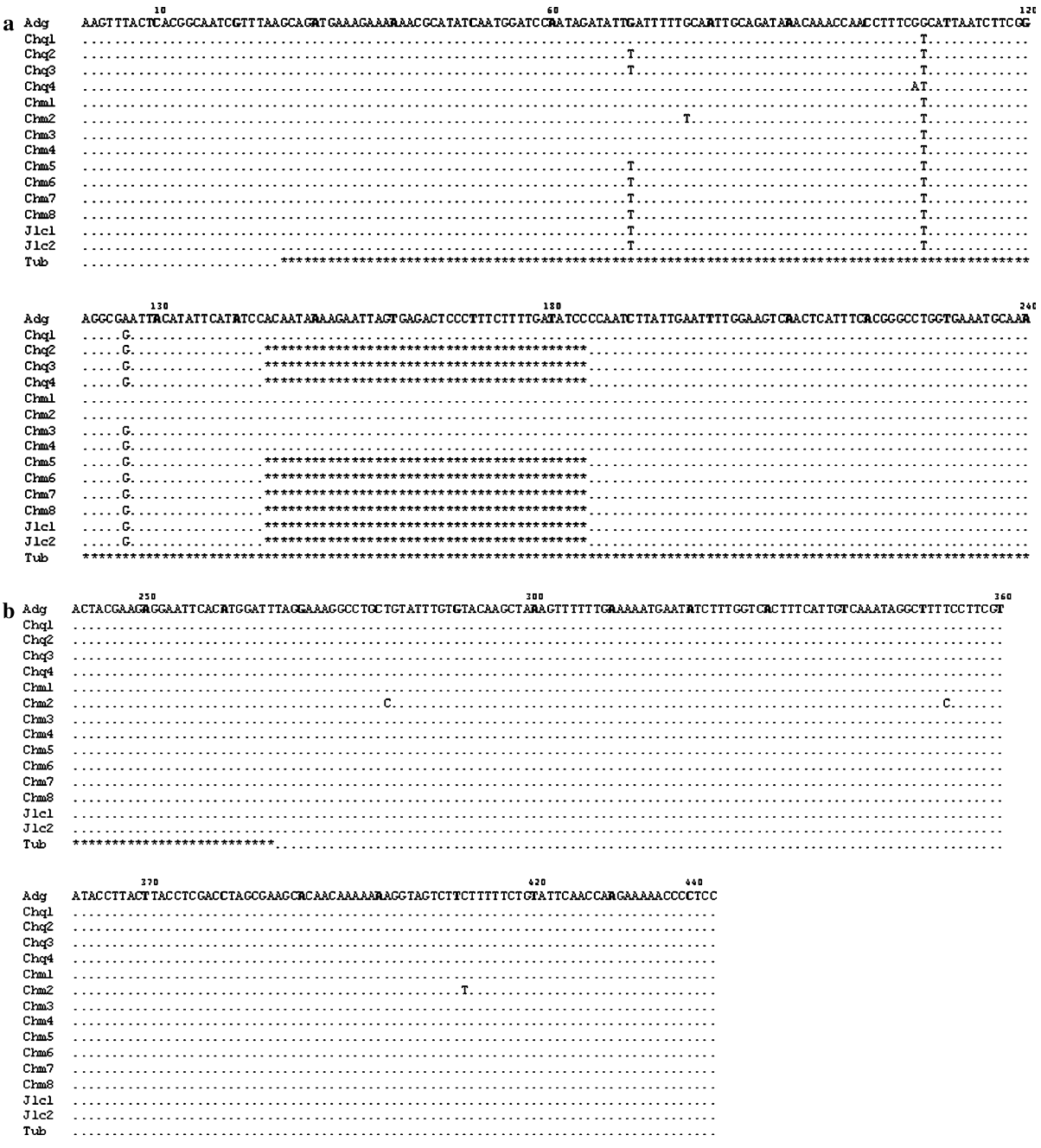

Fig. 3. Comparative sequences of 14 accessions of *Solanum chiquidenum*, *S. chomatophilum*, and *S. jalcae* chosen from throughout the range of these species, with nine accessions possessing an identical 41 bp deletion and five accessions lacking this deletion. These are compared to our new sequences of one accession each of *S. tuberosum* Chilotanum Group (from Chile, possessing a 241-bp deletion), and *S. tuberosum* Andigenum Group (lacking a deletion). The nucleotides that are homologous to the sequence of *S. tuberosum* Andigenum Group are indicated by periods, and deleted regions are indicated by asterisks. Accession codes are: adg, *S. tuberosum* Andigenum Group; chq1, *S. chiquidenum* OCHS 12566; chq2, SSTS 7331; chq3, OCHS 14482; chq4, OCH 11059; chm1, *S. chomatophilum* OCH 13325; chm2, SSTS 7341; chm3, OCH 11061; chm4, OCHS 12561; chm5, OCH 13208; chm6, SSTS 7327; chm7, OCH 13288; chm8, OCH 1512a; jlc1, *S. jalcae* OCHS 16021; jlc2, SSTS 7317; tub *S. tuberosum* Chilotanum Group. See Table 3 for locality data

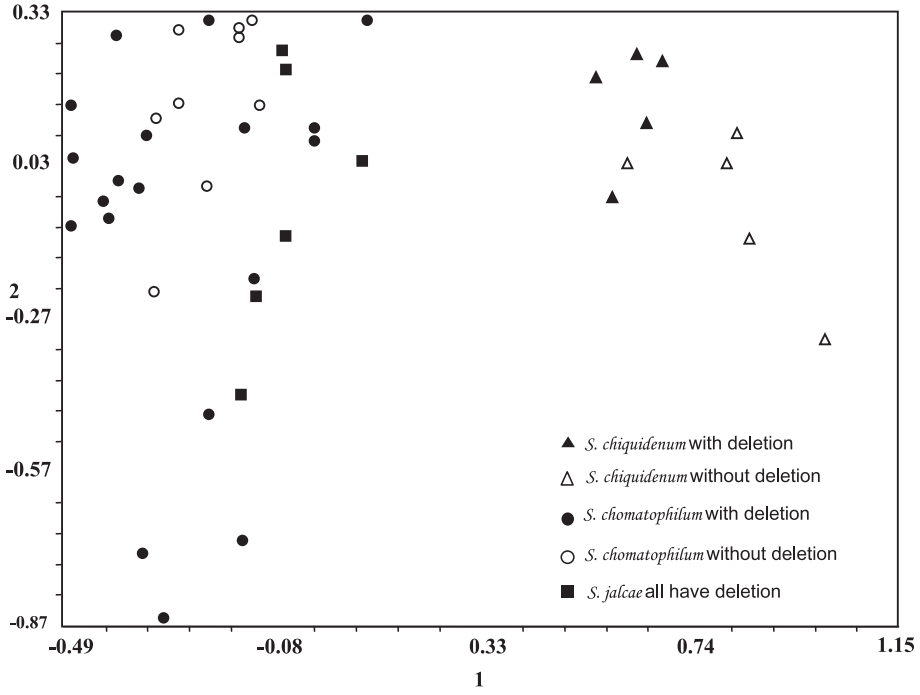

Fig. 4. Principal components analysis of 28 accessions *S. chomatophilum*, ten *S. chiquidenum*, and six *S. jalcae*, with the presence or absence of the 41 bp deletion in the *trn*V-UAC/*ndh*C intergenic spacer region indicated by closed or open symbols

Table 4. Alternative hypotheses of series affiliations of *S. chiquidenum*, *S. chomatophilum*, and *S. jalcae*

Species	Hypotheses of series affiliations			
	Correll 1962	Hawkes 1990	Ochoa 1999	Spooner and Salas 2006
Solanum chiquidenum Ochoa	*Piurana*	*Tuberosa*	*Piurana*	*Piurana*
S. chomatophilum Bitter	*Piurana*	*Conicibaccata*	*Conicibaccata*	*Piurana*
S. jalcae Ochoa	*Ingaefolia*	*Piurana*	*Ingifolia*	*Piurana*

Our study documents yet a third *ndh*C-*trn*V intergenic region deletion, of 41 bp, present in *S. chomatophilum*, *S. chiquidenum*, and *S. jalcae*. Like the two examples above, this deletion is present in only a subset of the populations of these species, and within a defined geographic range. There are three possible explanations for this pattern: 1) hybridization, 2) lineage sorting, and 3) parallel deletion events. Crossing barriers are poorly developed among many species in section *Petota*, providing many opportunities for natural interspecific hybridization. Hawkes (1990) speculates that many wild species had former separate distributions and came together through habitat disturbance caused by humans (Anderson's [1948] "hybridization of the habitat" hypothesis).

If hybridization is responsible for the spread of this 41 bp deletion in only some populations of these species, and if *S. chiquidenum* is not a sister species to *S. chomatophilum* + *S. jalcae* as a possible single species, it is a signal for frequent interspecific gene flow. Experimental crosses show that such hybridization is possible. Reciprocal crosses between *S. chiquidenum* and *S. chomatophilum* are successful although seed set is low. Reciprocal crosses between *S. chomatophilum* and *S. jalcae* are successful with high production of seeds.

However, the attempts to produce seeds from reciprocal crosses between *S. jalcae* and *S. chiquidenum* have failed (Ochoa 1990). Additional unpublished data from CIP show crosses of *S. chomatophilum* as the female parent with other species in the range of *S. chomatophilum, S. jalcae, and S. chiquidenum* (*S. anamatophilum* and *S. x blanco-galdosii*) to be successful with high seed production, but we did not find the deletion in these species.

Alternatively, lineage sorting explains shared polymorphism in different species as a result of incomplete sorting out of multiple alleles from a highly polymorphic progenitor species (Neigel and Avise 1986). The shared polymorphisms in our example, and in the *S. berthaultii*/*S. neorossii*/*S. tarijense* example, may be caused by lineage sorting. The resolution of these alternatives requires broader-scale phylogenetic analyses with multiple molecular markers, currently in progress. Once these results are obtained, the shared deletion mutations can be put in better perspective and contribute to our understanding of the taxonomic problems within the section.

A survey of the *ndh*C-*trn*V intergenic region in potato, tomato, and many other plants shows deletions to be common in this region. However, the specificity of the same 41 bp size deletion, to the exact region of plastid DNA, in the same geographic region, makes it unlikely that this is a parallel event.

Our results add another example to the phylogenetic utility of single and rare events. For example, Jansen and Palmer (1987) determined that the subtribe Barnadesiinae was the sister clade the rest of the Asteraceae through a single 22-kilobase-pair inversion in the plastid DNA. Yao-wu et al. (2006) determined, by a single retroposon insertion in the nuclear gene granule-bound starch synthase I (GBSSI or *waxy*), an ancient hybrid history and showed relationships of *Atropa belladonna*. Continued interspecies comparisons in potato, and among other closely related groups, surely will uncover more rare characters in intergenic spacers in the plastid genome.

We thank the staffs of the US Potato Genebank and the International Potato Center for germplasm, the International Potato Center for greenhouse facilities, John A. Lucey for advice on color conversions, and Flor Rodríguez for technical advice. This research was supported by the USDA, CIP, and by NSF DEB 0316614 to David Spooner entitled "A world-wide treatment of *Solanum* (http://www.nhm.ac.uk/research-curation/projects/solanaceaesource//)."

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