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Sukhotu, Thitaporn
Kamijima, Osamu
Hosaka, Kazuyoshi

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Chloroplast DNA variation in the most primitive cultivated diploid potato species *Solanum stenotomum* Juz. et Buk. and its putative wild ancestral species using high-resolution markers

Thitaporn Sukhotu[1], Osamu Kamijima[2] and Kazuyoshi Hosaka[3]*

*[1]Graduate School of Science and Technology, Kobe University, Rokkodai, Nada, Kobe 657-8501, Japan; [2]Faculty of Agriculture, Kobe University, Rokkodai, Nada, Kobe 657-8501; [3]Food Resources Education and Research Center, Kobe University, 1348 Uzurano, Kasai, Hyogo 675-2103, Japan; *Author for correspondence (e-mail: hosaka@kobe-u.ac.jp)*

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Abstract

Solanum stenotomum Juz. et Buk. (2*n*=2*x*=24) is considered to be the most primitive diploid cultivated species from which all the other Andean cultivated potatoes were originated (Hawkes 1990). To disclose chloroplast DNA (ctDNA) variability and the maternal origin of *S. stenotomum*, 36 accessions of *S. stenotomum* and 86 accessions of putative wild ancestral species were determined for ctDNA types and analyzed by high-resolution markers (seven ctDNA microsatellites and an H3 marker). High-resolution markers discriminated 57 different ctDNAs (haplotypes), which were classified into the W-type ctDNA group and C-, S- and A-type ctDNA group, and within the latter group S- and A-type ctDNAs were unrelated from each other among many different haplotypes mostly having C-type ctDNA. This ctDNA relationship supported our previous findings obtained for mostly Andean cultivated species (Sukhotu et al. 2004). Compared with other putative ancestral wild species, *S. stenotomum* showed somewhat limited ctDNA diversity, having two major haplotypes 1 and 2 also

found in different wild species in different places. Therefore, the ctDNA in *S. stenotomum* was of at least dual origins either by successive domestication from different species or else by introgression after initial *S. stenotomum* arose.

Introduction

Potato and its cultivated and wild relatives (tuber-bearing *Solanum* species) are classified into seven cultivated and 226 wild species (Hawkes 1990) or one cultivated and 199 wild species (Spooner and Hijmans 2001; Huamán and Spooner 2002) in the genus *Solanum* L. sect. *Petota* Dumortier. *Solanum stenotomum* Juz. et Buk. (or *S. tuberosum* Group Stenotomum by Huamán and Spooner 2002) ($2n=2x=24$) is highly variable (Hawkes 1956, 1990; Bukasov 1978; Ochoa 1990) and is thought to be the most primitive diploid cultivated species from which all the other Andean cultivated potatoes were originated (Hawkes 1990). It is still grown by native farmers from central Peru to central Bolivia in the Andean highlands, planted together with other cultivated potato

species, particularly with the most important Andean cultivated species *S. tuberosum* ssp. *andigena* (2*n*=4*x*=48).

There have long been arguments over which wild species gave rise to *S. stenotomum*. From the morphological and phytogeographical points of view, Hawkes (1958) suggested *S. leptophyes* and *S. canasense* as the ancestral species of *S. stenotomum* and later, favored *S. leptophyes* because it is distributed at the same altitude and phytogeographical region as *S. stenotomum* (Hawkes 1988, 1990; Hawkes and Hjerting 1989). As *S. stenotomum* is highly polymorphic, Ugent (1970) proposed its ancestor to be a single superspecies, the '*S. brevicaule* complex', which included *S. brevicaule*, *S. bukasovii*, *S. canasense*, *S. coelestipetalum*, *S. gourlayi*, S. *leptophyes*, *S. multidissectum*, *S. multiinterruptum* and *S. spegazzinii*. Bukasov (1966, 1978) also claimed *S. canasense* and *S. leptophyes* (Bukasov 1966), or *S. brevicaule*, *S. bukasovii*, *S. candolleanum*, *S. leptophyes* and *S. sparsipilum* (Bukasov 1978) for the origin of *S. stenotomum*. Ochoa (1990) proposed *S. brevicaule*, *S. bukasovii* and *S. canasense* for ancestral species of *S. stenotomum*. Most of these wild species are closely related to each other and there are many controversies in their taxonomy (Correll 1962; Bukasov 1978; Hawkes 1990; Ochoa 1990;

van den Berg et al. 1998; Miller and Spooner 1999). RFLP analyses of nuclear DNA supported close relationships among *S. stenotomum, S. bukasovii, S. canasense* and *S. tuberosum* spp. *andigena* (Bonierbale et al. 1990; Debener et al. 1990; Miller and Spooner 1999), although AFLP analysis by Kardolus et al. (1998) showed slightly different relationships; *S. canasense, S. multidissectum* and *S. tuberosum* ssp. *tuberosum* in one group *and S. stenotomum* and *S. brevicaule* in another group.

However, none of the experimental data have tested hypotheses on the origin of *S. stenotomum* except chloroplast DNA (ctDNA) RFLP data. CtDNA is inherited maternally in most angiosperms and evolves relatively slowly: therefore, it can be a reliable indicator to trace maternal ancestry of crops (Palmer et al. 1988). CtDNA RFLP analyses were used to evaluate genetic diversity in the Andean cultivated and closely related wild species (Hosaka et al. 1984; Buckner and Hyde 1985; Hosaka 1986, 1995; Hosaka and Hanneman 1988a, 1988b). Wide ctDNA diversity, namely five basic ctDNA types (W, T, C, S and A types) (Hosaka 1986), was found within *S. tuberosum* ssp. *andigena* (Hosaka and Hanneman 1988a). These types of ctDNA, except T-type ctDNA, were shared with *S. stenotomum* and with

putative ancestral species of *S. stenotomum*. (Hosaka 1995). (In Hosaka 1995, one accession of *S. stenotomum* was reported to have T-type ctDNA, but it was confirmed not to be *S. stenotomum* by Hosaka 2004). The T-type ctDNA, predominant in the common potato (*S. tuberosum* ssp. *tuberosum*), was incorporated from some populations of *S. tarijense* into *S. tuberosum* ssp. *andigena* during its migration to southern coastal Chile (Hosaka 2003, 2004). The wide ctDNA diversity among the Andean cultivated and closely related wild species led to a hypothesis of multiple origins for *S. stenotomum*, by successive domestications temporally and spatially from the 'ancestral species' complex (referred to as the successive domestication hypothesis) (Hosaka 1995).

Recently, several high-resolution markers have become available to detect ctDNA variation. CtDNA microsatellite markers detect polymorphisms in repeated numbers of mononucleotides in ctDNA (Provan et al. 2001), which revealed much higher levels of diversity than ctDNA RFLPs (Powell et al. 1995; Bryan et al. 1999; Provan et al. 1999; Ishii and McCouch 2000; Xu et al. 2002; Hosaka 2003). Due to the haploid nature and high copy number of the

chloroplast genome, the ctDNA microsatellites can be easily analyzed by PCR.

In a previous study (Sukhotu et al. 2004), 75 accessions of all seven Andean cultivated species (including 10 accessions of *S. stenotomum*) and 16 accessions of nine wild species were analyzed by using ctDNA microsatellite markers. Twenty-five different ctDNAs (=haplotypes) found among them revealed diverse differentiation within and between W-type ctDNA and S-, C- and A-type ctDNAs. T-type ctDNA and S- and A-type ctDNAs were clearly distinguished from the group of haplotypes having W- and C-type ctDNAs, respectively. Interestingly, the two most frequent haplotypes in *S. stenotomum*, and in *S. tuberosum* ssp. *andigena* as well, were not found in any wild species (Sukhotu et al. 2004). In this study, we analyzed a large number of accessions of *S. stenotomum* and its presumed ancestral wild species using the same set of high-resolution ctDNA markers. The aims of this research are 1) to evaluate whether the relationships of ctDNA types shown by high-resolution markers mostly among the Andean cultivated species in the previous study can also be supported among the most primitive cultivated species *S. stenotomum* and its putative ancestral species, 2) to reveal ctDNA variability in *S. stenotomum* compared with

ancestral wild species, 3) to disclose (a) donor species of the most frequent ctDNA in Andean potatoes and to discuss the origin of *S. stenotomum*.

Materials and methods

Plant material and DNA isolation

Thirty-six accessions of *S. stenotomum*, 78 accessions of putative wild ancestral species and two accessions each of *S. chacoense*, *S. megistacrolobum*, *S. sparsipilum*, and *S. vernei* were used in this study (Table 1). The accessions with CIP numbers and PI numbers were obtained as seeds from the International Potato Center, Lima, Peru and the Potato Introduction Station (NRSP-6), Sturgeon Bay, Wisconsin, USA, respectively. CtDNA was isolated by the method of Hosaka and Hanneman (1987) from fresh leaves collected from bulked samples of more than ten seedlings except for a few accessions isolated from one seedling.

CtDNA analysis

CtDNA types were determined based on restriction fragment patterns of *Bam*HI, *Hin*dIII or *Pvu*II, as described by Hosaka and Hanneman (1988b). Seven ctDNA microsatellite markers, developed by Provan et al. (1999) from tobacco ctDNA (NTCP markers), and H3 marker (Hosaka 2003) were used to analyze potato ctDNA in this study. The H3 marker was a PCR-based marker, which amplified the coding regions of *ycf4* and *ycf10* of the ctDNA. Polymorphisms were detected after restriction digestion of PCR products by *Dra*I restriction enzyme. Experimental procedures were all described previously (Sukhotu et al. 2004).

Data analysis

CtDNA microsatellite markers were scored as presence (1) or absence (0) of each fragment. The extent of genetic variation in a species was measured by average gene diversity (Nei and Kumar 2000). The gene diversity at a locus is defined as

$$h = 1 - \sum_{i=1}^{q} x_i^2$$

where x_i is the frequency of the *i*-th allele and *q* is the number of alleles. Average gene diversity (*H*) is the average of this quantity over all loci. The difference in *H* between the two species was tested by a *t* test. Gene diversity (*h*) based on frequencies of ctDNA types or haplotypes were also calculated.

Pairwise distances, shown as total character differences between accessions, were obtained using ctDNA microsatellites and H3 marker. The un-weighted pair-group method with arithmetic means (UPGMA) was used for clustering. To search for the most probable UPGMA dendrogram, 1,000 bootstraps were carried out and an unrooted phylogram was obtained using PAUP 4.0b10.

Results

CtDNA types

Restriction fragment patterns obtained in this study by digestion with *Bam*HI, *Hin*dIII or *Pvu*II were all similar to those reported earlier (Hosaka and Hanneman 1988b); thus, known ctDNA types could be assigned to all accessions used (Table 1). *S. brevicaule*, *S. vernei*, and

S. sparsipilum were monomorphic with W-type ctDNA and *S. megistacrolobum* with C-type ctDNA. All the other wild species were polymorphic with W-, C-, S- or A-type ctDNA, among which W and C were major ctDNA types. Types A and W2 ctDNA were found in one accession each of *S. bukasovii* and *S. leptophyes*, respectively. In the accessions of *S. stenotomum*, W-, A-, C- and S-type ctDNAs were found, as reported previously (Hosaka 1995). Major types were S (69.4%) and A (22.2%).

For the accessions of wild species, W-type ctDNA was predominantly distributed in Bolivia or Argentina (92.3%), while C-, S- and A-type ctDNAs in Peru (91.4%) (Table 1). In *S. stenotomum*, S-type ctDNA was widely spread in the distributional area, indicating the frequent exchange of cultivated materials by human beings. However, A-type ctDNA tended to be localized into central Peru, and W-type ctDNA in the peripheral areas of its distribution (Fig. 1).

Polymorphisms with ctDNA microsatellites and H3 marker.

Seven ctDNA microsatellites produced 43 fragments containing 10 new fragments (shown in Italic) compared with our previous study (Sukhotu et al. 2004): NTCP6 (127, *171*, 172, 173, 174, 175 and *176* base pair

fragments), NTCP7 (*172*, 173 and 174), NTCP8 (249, 250, 251, 252, 253 and *254*), NTCP9 (247, *248*, *250*, *258*, 259, *261*, 279, 288, 289 and 317), NTCP12 (234, 235, 236, 237, 238 and 239), NTCP14 (149, 150, 151, 152, 153 and *154*), and NTCP18 (186, 187, 188, *189* and 196). The H3 marker produced two types of restriction banding patterns (types 1 and 2, see Sukhotu et al. 2004).

Out of 45 fragments and types, 24 were shared between *S. stenotomum* and wild species. Twenty fragments were specifically found in wild species of which nine were unique to single accessions. One fragment (172 bp of NTCP7) was unique to one accession of *S. stenotomum,* which had W-type ctDNA. The 127 bp fragment of NTCP6 was correlated with S-type ctDNA in *S. stenotomum,* but not always in wild species. The 239 bp fragment of NTCP12 was correlated with S-type ctDNA except in one accession of *S. multidissectum*. Theses two fragments were perfectly correlated with S-type ctDNA in the previous study (Sukhotu et al. 2004). No other correlation between specific microsatellite fragments or H3 types and ctDNA types was found (Table 1).

The 43 microsatellite fragments and two H3 types discriminated 57 different ctDNAs or haplotypes, among which 52 were

found in wild species, seven found in *S. stenotomum* and two shared between them (Table 1). Thirty-seven (43.0%) of 86 accessions of wild species and four (11.1%) of 36 accessions of *S. stenotomum* were uniquely distinguished. Haplotypes 1 (16.7%) and 2 (66.7%) predominated amongst *S. stenotomum* accessions.

Genetic diversity in S. stenotomum and its wild ancestral species

Twenty-five different microsatellite fragments and H3 marker types, or 7 haplotypes were detected in *S. stenotomum*, which gave 0.69 markers per accession or 0.19 haplotypes per accession being much lower than those of any putative ancestral wild species. Gene diversity (*h*) in *S. stenotomum* ranged from 0.10 (NTCP9 and NTCP18) to 0.47 (NTCP6) depending on microsatellite loci. The average gene diversity (*H*) was 0.31 in *S. stenotomum*, which was significantly lower than those of *S. canasense*, *S. leptophyes*, '*S. brevicaule* complex', and all wild species accessions as a group (Table 2). Consequently, gene diversity based on haplotypes showed a lower value in *S. stenotomum* (*h*=0.52) than those of any wild species. In contrast, gene diversity based on ctDNA types in *S. stenotomum* (*h*=0.46) was higher than those of *S. brevicaule*,

S. bukasovii and *S. candolleanum*, and almost similar to those of *S. canasense*, *S. leptophyes* and *S. multidissectum*.

Relationship between haplotypes and ctDNA types

A UPGMA consensus tree based on 1,000 bootstrap replicates was obtained to show similarities among these haplotypes (Fig. 2). This unrooted tree could be separated to two halves based on ctDNA types: a group of haplotypes having W- or W2-type ctDNA and another with A, S or C-type ctDNA. In the A, S or C-type ctDNA group, 31 haplotypes showed C-type ctDNA. The A-type ctDNA was found in haplotypes 1, 20 and 22, which formed a cluster together with some other related haplotypes. The S-type ctDNA formed a specific cluster with haplotypes 2, 14 and 56, and also found as haplotypes 25 and 51 in somewhat unrelated clusters.

Species relationships

Since the UPGMA tree showed clear separation between W- and W2-type ctDNA and the others, accessions of any one species having both W-type and C-type ctDNA in a species were clustered separately (*S. canasense*, *S. leptophyes* and *S. stenotomum*). Most wild species

accessions had unique haplotypes in different clusters, except for *S. bukasovii*, *S. canasense*, *S. candolleanum* and *S. multidissectum* that often had haplotypes shared with each other. Consequently, none of species formed a distinct cluster (Fig. 2).

Although only seven haplotypes were found in *S. stenotomum*, these haplotypes were found in different clusters in Fig. 2. The most frequent haplotype 2 in *S. stenotomum* was shared with six accessions of *S. canasense* and one each of *S. candolleanum* and *S. multidissectum*. The haplotype 2 was further clustered with haplotype 14 of *S. bukasovii* and 56 of *S. stenotomum* and formed Cluster A in Fig. 2. Collection sites of the wild species accessions in Cluster A were plotted on a distribution map, showing limited localization in southern Peru or near Lake Titicaca (Fig. 1). The second and third major haplotypes 1 and 22, or A-type ctDNA in *S. stenotomum* were clustered together mostly with accessions of *S. bukasovii* (Cluster B). The haplotype 1 was not found in any wild species, but haplotype 22 was shared with an accession of *S. bukasovii*. The accessions of wild species in Cluster B were distributed from central Peru to northern Bolivia, of which A-type ctDNA was distributed in the central Peru (Fig. 1).

Discussion

CtDNA differentiation

In contrast to five ctDNA types distinguished by restriction site differences, 57 haplotypes were distinguished by ctDNA microsatellites and H3 marker with the same set of samples. This higher resolving power is probably due to a higher mutation rate of repeat numbers in the simple sequence repeated regions than the rate of base substitution and insertion/deletion events in the chloroplast genome (Ishii et al. 2001).

CtDNAs in 36 accessions of *S. stenotomum* and 86 accessions of 11 wild species were classified into the W-type ctDNA group and C-, S- and A-type ctDNA group, and within the latter group S- and A-type ctDNA were separated distinctly from each other among many different haplotypes mostly having C-type ctDNA (Fig. 2). This relationship among ctDNA types based on haplotype differences is in good agreement with the previous results obtained for 75 accessions of seven cultivated species and 16 accessions of nine wild species (Sukhotu et al. 2004), and thus, can be widely accepted in the cultivated potato species and their closely related species as well.

Species differentiation

None of species was uniquely identified either by ctDNA types or ctDNA microsatellites (Fig. 2) and each putative ancestral species contained a relatively high degree of gene diversity (Table 2). We further found that *S. bukasovii, S. canasense, S. candolleanum* and *S. multidissectum* often had the same haplotypes. It has been known that *S. bukasovii, S. canasense* and *S. multidissectum* are highly variable (Hawkes 1990), and they were considered as a single species by Ochoa (1992). The *S. brevicaule* complex (including approximately 30 taxa) was investigated extensively morphologically by van den Berg et al. (1996, 1998), but none of the taxa within the complex was uniquely distinguished. Recently, Miller and Spooner (1999) studied the *S. brevicaule* complex using nuclear RFLPs and RAPDs and separated members of the complex only weakly into 1) the northwestern Bolivian and Argentine taxa, and 2) the Peruvian and adjacent northwestern Bolivian accessions with most of the cultigens. They suggested that most of the 30 taxa of the *S. brevicaule* complex are artificial, and should be treated as a single highly polymorphic species *S. brevicaule* (Miller and Spooner 1999). These suggest that the overlapping variation in ctDNA among these putative ancestral wild species is due to

frequent hybridization and introgression (Hawkes 1990), poor species differentiation of the taxa with common ancestry (van den Berg et al. 1998; Miller and Spooner 1999), or both.

Domestication of S. stenotomum

The most primitive cultivated diploid species, *S. stenotomum*, had only seven out of the 57 haplotypes identified in this study, of which two (haplotypes 1 and 2) occupied 83.3% of *S. stenotomum* accessions. Consequently, gene diversity based on haplotypes in *S. stenotomum* was lower than that of any putative ancestral species (Table 2). This result contrasts with our previous understanding on ctDNA diversity in *S. stenotomum*. As seen in that study of gene diversity (*h*) based on ctDNA (Table 2), we believed *S. stenotomum* had wider intraspecific ctDNA diversity than any one putative ancestral species and therefore we proposed a hypothesis of multiple origins for *S. stenotomum* by successive domestication from the 'ancestral species' complex (the successive domestication hypothesis) (Hosaka 1995). The present study using high-resolution markers does not necessarily support the successive domestication hypothesis. Somewhat limited ctDNA variation in *S. stenotomum* could be derived by differentiation or

domestication from (an) ancestral wild species with a larger variation range. Our study indicated two major ctDNAs in *S. stenotomum*; haplotype 1 (or A-type ctDNA) and haplotype 2 (or S-type ctDNA), which were also found in *S. bukasovii* in central Peru and in *S. canasense*, *S. candolleanum* or *S. multidissectum* in southern Peru, respectively. Thus, it can be suggested that ctDNA in *S. stenotomum* was at least of dual origins because different wild species, or varieties according to Ochoa (1992), and different originating locations were involved. However, it is not clear whether the ctDNA diversity in *S. stenotomum* was attributed to domestication itself from different ancestral species (=the successive domestication hypothesis) or to later hybridization after *S. stenotomum* was initially domesticated (referred to as the introgression hypothesis). Occurrence of natural hybridization of *S. stenotomum* with wild diploid species has been known as a typical example in *S. ajanhuiri*, a cultivated diploid species of hybrid origin between *S. stenotomum* and *S. megistacrolobum* (Huamán et al. 1982, Johns and Keen 1986), and also with *S. sparsipilum* (Ugent 1970; Rabinowitz et al. 1990).

The seven haplotypes found in *S. stenotomum* might have rather diverse origins as shown in their different clusters in Fig. 2.

Haplotypes 55 and 57 showed W-type ctDNA and were collected from the extreme north and south ends of its distributional area (Fig. 1). According to the successive domestication hypothesis, *S. stenotomum* accessions having W-type ctDNA are the most primitive varieties first domesticated from the 'ancestral species' complex (Hosaka 1995). Alternatively, these accessions might be of hybrid origin with wild species having W-type ctDNA because these haplotypes were very rare in this study and were found only in these extreme areas. Additional possibilities such as seed contamination, crossing mistakes or identification errors may be possible since these *S. stenotomum* accessions were obtained as botanical seeds.

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Legend of Figures

Figure 1. Maps showing approximate collection sites of the accessions used. *S. stenotomum* accessions are shown by their ctDNA types.

Figure 2. Unrooted UPGMA consensus tree based on 1000 bootstraps, showing haplotype similarity. CtDNA types and species names are also shown with haplotype numbers. *S. stenotomum* accessions are shown in Bold. When multiple accessions had the same haplotypes, the number of accessions is prefixed to the species abbreviation. See Table 1 for species abbreviation.

Table 1. *Solanum* species accessions used in this study and their chloroplast DNA (ctDNA) types, microsatellite marker phenotypes (in base pairs), H3 types and haplotypes.

Species and accession	Locality*	CtDNA type	Microsatellite marker (NTCP) phenotype							H3 type	Haplo-type
			6	7	8	9	12	14	18		
Series *Yungasensa*											
S. chacoense Bitt. (chc)											
PI 537025	B, Chuquisaca	W	172	174	252	279	235	149	188	1	3
chc525-3	?	W	175	174	252	279	234	152	187	1	4
Series *Megistacroloba*											
S. megistacrolobum Bitt. ssp. *megistacrolobum* (mga)											
PI 473361	B, La Paz	C	175	174	250	288	237	150	186	1	5
PI 473356	B, Potosi	C	173	173	249	289	237	152	186	1	6

Series *Tuberosa*

S. brevicaule Bitt. (brc)

PI 498218	B, La Paz	W	172	174	252	247	236	153	187	1	7
PI 545970	B, La Paz	W	172	173	252	247	236	151	186	1	8
PI 310929	B, Cochabamba	W	172	173	253	248	236	153	186	1	9
PI 310930	B, Cochabamba	W	172	173	253	248	236	153	186	1	9
PI 473378	B, Cochabamba	W	172	173	253	247	236	152	186	1	10
PI 498110	B, Cochabamba	W	172	173	253	248	236	153	186	1	9
PI 498111	B, Cochabamba	W	172	173	253	248	236	152	186	1	11
PI 498112	B, Cochabamba	W	172	173	253	248	236	152	186	1	11
PI 498113	B, Cochabamba	W	172	173	253	247	236	152	186	1	10
PI 498114	B, Cochabamba	W	172	173	253	247	236	152	186	1	10
PI 545968	B, Cochabamba	W	172	173	253	247	236	152	186	1	10

PI 545971	B, Cochabamba	W	172	173	253	247	236	151	186	1	12
S. bukasovii Juz. (buk)											
PI 275271	P, Huanuco	C	174	174	251	289	237	151	186	1	13
PI 365318	P, Huanuco	S	127	173	252	289	239	150	186	2	14
PI 365321	P, Huanuco	C	173	174	251	289	237	150	186	1	15
PI 365304	P, Lima	C	173	174	251	289	237	151	186	2	16
PI 210042	P, Junin	C	176	174	251	289	237	150	186	1	17
PI 210051	P, Junin	C	174	174	251	289	237	151	186	1	13
PI 498219	P, Junin	C	174	173	251	289	237	150	186	1	18
PI 498220	P, Junin	C	176	174	250	289	237	150	186	1	19
PI 498221	P, Junin	C	176	174	250	289	237	150	186	1	19
PI 473492	P, Huancavelica	A	174	174	250	289	237	150	186	2	20

PI 473493	P, Huancavelica	C	175	174	251	258	237	149	186	2	21
PI 473450	P, Ayacucho	C	174	174	251	289	237	151	186	2	22
PI 473453	P, Ayacucho	C	175	174	251	258	237	149	186	2	21
PI 442698	P, Cuzco	C	173	174	253	289	235	150	186	2	23
PI 473469	P, Cuzco	C	174	174	252	259	236	149	186	2	24
PI 473491	P, Cuzco	S	173	174	250	250	239	150	186	1	25
PI 414155	P, Apurimac	C	175	174	253	289	237	151	186	2	26
PI 458379	P, Apurimac	C	174	174	252	289	236	151	186	2	27
PI 473447	P, Arequipa	C	174	174	253	289	236	150	186	2	28
PI 473452	P, ?	C	175	174	251	258	237	149	186	2	21
S. canasense Hawkes (can)											
PI 473355	P, Ayacucho	C	173	174	252	289	235	152	186	1	30

PI 210035	P, Cuzco	C	173	174	250	289	236	150	186	2	31
PI 246533	P, Cuzco	C	174	174	252	259	236	149	186	1	32
PI 265864	P, Cuzco	C	174	174	252	259	236	149	186	1	32
PI 265875	P, Cuzco	C	174	174	252	259	236	149	186	1	32
PI 283074	P, Cuzco	C	174	174	252	289	236	150	186	2	33
PI 283080	P, Cuzco	C	176	174	251	261	238	150	187	1	34
PI 310937	P, Cuzco	C	174	174	252	259	236	149	186	1	32
PI 310938	P, Cuzco	C	174	174	252	259	236	149	186	2	24
PI 310939	P, Cuzco	C	174	174	252	259	236	149	186	2	24
PI 310940	P, Cuzco	C	174	174	252	259	236	149	186	2	24
PI 310941	P, Cuzco	C	174	174	252	259	236	149	186	2	24
PI 473348	P, Cuzco	C	176	174	251	259	238	150	187	1	35
PI 230511	P, Puno	S	127	173	251	289	239	150	186	2	2

PI 265863	P, Puno	S	127	173	251	289	239	150	186	2	2
PI 283084	P, Puno	S	127	173	251	289	239	150	186	2	2
PI 310956	P, Puno	S	127	173	251	289	239	150	186	2	2
PI 442695	P, Puno	S	127	173	251	289	239	150	186	2	2
PI 442696	P, Puno	C	174	174	251	289	237	150	186	2	36
PI 458377	P, Puno	C	175	173	250	289	236	150	186	2	37
PI 473345	P, Puno	S	127	173	251	289	239	150	186	2	2
PI 473346	P, Puno	C	173	174	250	317	236	151	186	2	38
PI 265865	B, La Paz	W	173	173	253	247	236	151	186	1	29
S. candolleanum Berth. (cnd)											
PI 545972	B, La Paz	C	174	174	251	289	237	150	186	2	36
PI 498226	B, La Paz	S	127	173	251	289	239	150	186	2	2

PI 498313	B, Oruro	C	175	174	251	289	236	151	186	2	39
S. coelestipetalum Vargas (cop)											
PI 473354	P, Apurimac	C	175	174	250	289	237	151	186	2	40
S. leptophyes Bitt. (lph)											
PI 473451	P, Ayacucho	C	175	174	251	258	237	150	186	2	41
PI 473445	P, Cuzco	C	174	174	251	259	236	150	196	2	42
PI 473448	P, Apurimac	C	176	174	251	259	237	151	186	2	43
PI 458378	P, Puno	W	172	174	252	247	236	152	187	1	44
PI 320340	B, Oruro	W	173	173	253	247	236	151	186	1	29
PI 545984	B, Oruro	W	173	173	253	247	236	151	186	1	29
PI 473495	B, Potosi	W	173	173	254	247	236	153	187	1	45
PI 545895	B, Potosi	W2	171	174	251	279	235	149	189	1	46

PI 545992	B, Potosi	W	172	173	252	247	236	152	186	1	47
PI 545994	B, Potosi	W	172	173	253	247	236	150	186	1	48
PI 545995	B, Potosi	W	173	174	252	248	236	154	186	1	49
PI 545997	B, Potosi	W	172	173	253	247	236	152	186	1	10
S. multidissectum Hawkes (mlt)											
PI 210044	P, Junin	C	174	173	251	289	237	150	186	2	50
PI 210055	P, Cuzco	S	173	174	250	250	239	150	186	1	25
PI 473349	P, Cuzco	C	173	174	250	289	236	150	186	2	31
PI 473353	P, Cuzco	S	174	174	251	250	238	151	186	1	51
PI 498304	P, Cuzco	S	173	174	250	250	239	150	186	1	25
PI 310955	P, Puno	S	127	173	251	289	239	150	186	2	2
PI 473352	P, Puno	S	173	174	250	250	239	150	186	1	25

S. sparsipilum (Bitt.) Juz. et Buk. (spl)

PI 498305	P, Cuzco	W	172	173	253	247	236	152	186	1	10
PI 498284	B, La Paz	W	172	174	252	247	236	152	187	1	44

S. vernei Bitt. et Wittm. ssp. *vernei* (vrn)

PI 545884	B, Cochabamba	W	172	173	253	247	237	152	186	1	52
PI 473308	A, Tucuman	W	175	174	253	279	235	150	187	1	53

S. stenotomum Juz. et Buk. ssp. *stenotomum* (stn)

CIP 703317	P, Ancash	S	127	173	251	289	239	150	186	2	2
CIP 702464	P, Huanuco	S	127	173	251	289	239	150	186	2	2
CIP 703843	P, Huanuco	A	174	174	251	289	237	151	186	2	22
CIP 700348	P, Junin	S	127	173	251	289	239	150	186	2	2
CIP 700670	P, Junin	S	127	173	251	289	239	150	186	2	2

CIP 701165	P, Junin	S	127	173	251	289	239	150	186	2	2
CIP 701960	P, Junin	A	174	174	250	289	237	151	186	2	1
CIP 701985	P, Junin	S	127	173	251	289	239	150	186	2	2
CIP 702033	P, Junin	A	174	174	250	289	237	151	186	2	1
CIP 702172	P, Junin	A	174	174	250	289	237	151	186	2	1
CIP 703034	P, Junin	S	127	173	251	289	239	150	186	2	2
CIP 703088	P, Junin	C	173	174	251	289	236	150	187	2	54
CIP 703151	P, Junin	S	127	173	251	289	239	150	186	2	2
CIP 703313	P, Junin	A	174	174	250	289	237	151	186	2	1
CIP 703698	P, Junin	S	127	173	251	289	239	150	186	2	2
CIP 703707	P, Junin	S	127	173	251	289	239	150	186	2	2
CIP 703708	P, Junin	W	176	174	254	247	235	149	187	1	55
CIP 703197	P, Huancavelica	S	127	173	251	289	239	150	186	2	2

CIP 703311	P, Huancavelica	S	127	173	251	289	239	150	186	2	2
CIP 702243	P, Ayacucho	A	174	174	250	289	237	151	186	2	1
CIP 702249	P, Ayacucho	S	127	173	251	289	239	150	186	2	2
CIP 703470	P, Ayacucho	S	127	173	251	289	239	150	186	2	2
CIP 702199	P, Cuzco	S	127	173	251	289	239	150	186	2	2
CIP 702353	P, Cuzco	S	127	173	251	289	239	150	186	2	2
CIP 703287	P, Cuzco	S	127	173	251	289	239	150	186	2	2
CIP 703933	P, Cuzco	A	174	174	251	289	237	151	186	2	22
CIP 702834	P, Puno	S	127	173	251	289	239	150	186	2	2
CIP 703319	P, Puno	S	127	173	251	289	239	150	186	2	2
CIP 703624	P, Puno	S	127	173	251	289	239	150	186	2	2
CIP 703637	P, Puno	S	127	173	251	289	239	150	186	1	56
CIP 700235	P, ?	S	127	173	251	289	239	150	186	2	2

CIP 703286	B, La Paz	S	127	173	251	289	239	150	186	2	2
CIP 703473	B, La Paz	A	174	174	250	289	237	151	186	2	1
CIP 702286	B, Cochabamba	S	127	173	251	289	239	150	186	2	2
CIP 702547	B, Potosi	S	127	173	251	289	239	150	186	2	2
CIP 702583	B, Potosi	W	172	172	253	247	236	151	186	1	57

*A Argentina, B Bolivia, P Peru

See Hawkes (1990) for species name abbreviation in parentheses

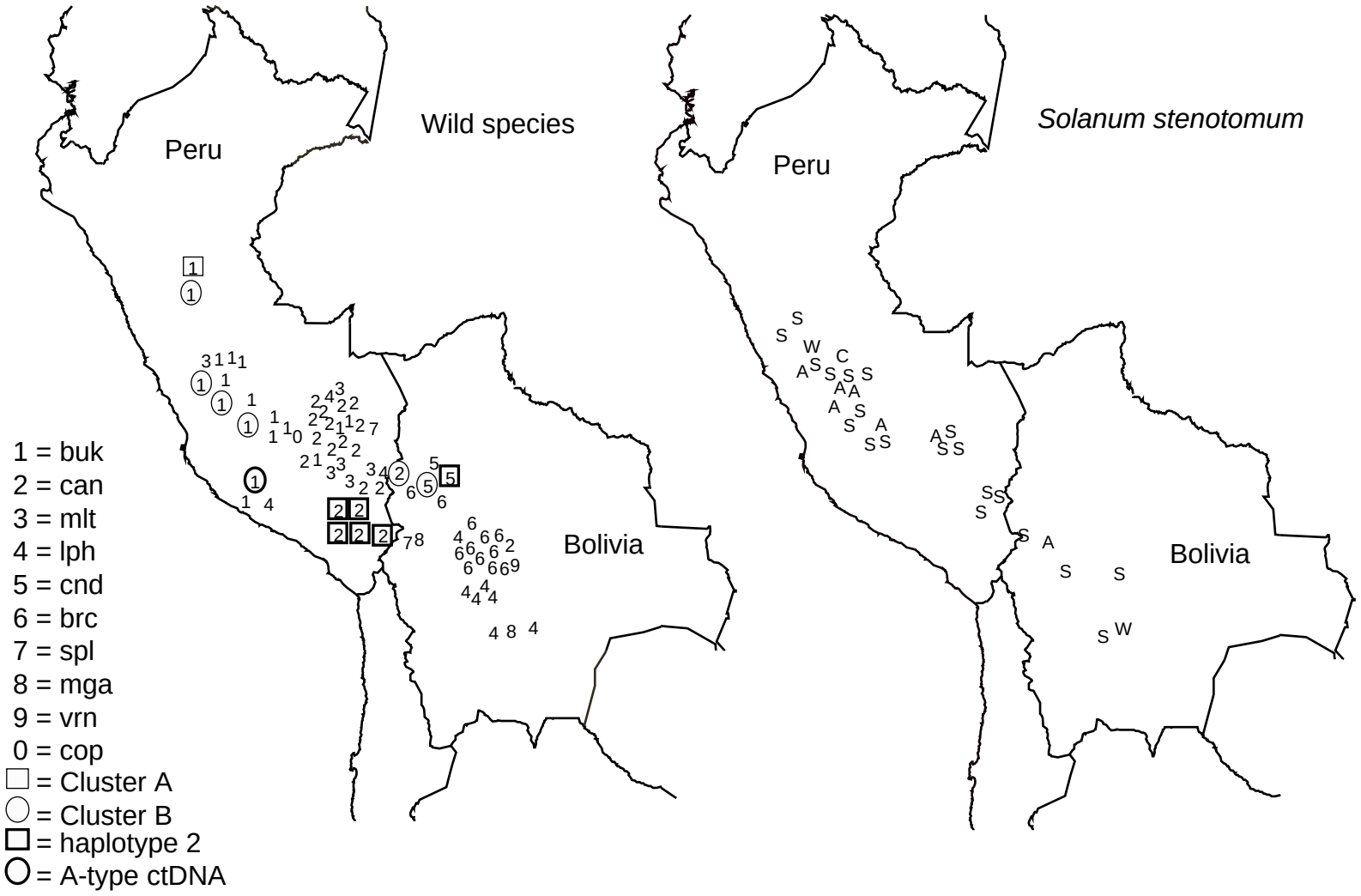
Table 2. Average gene diversity (*H*) based on ctDNA microsatellites (including H3 marker) and gene diversity (*h*) based on ctDNA types and haplotypes.

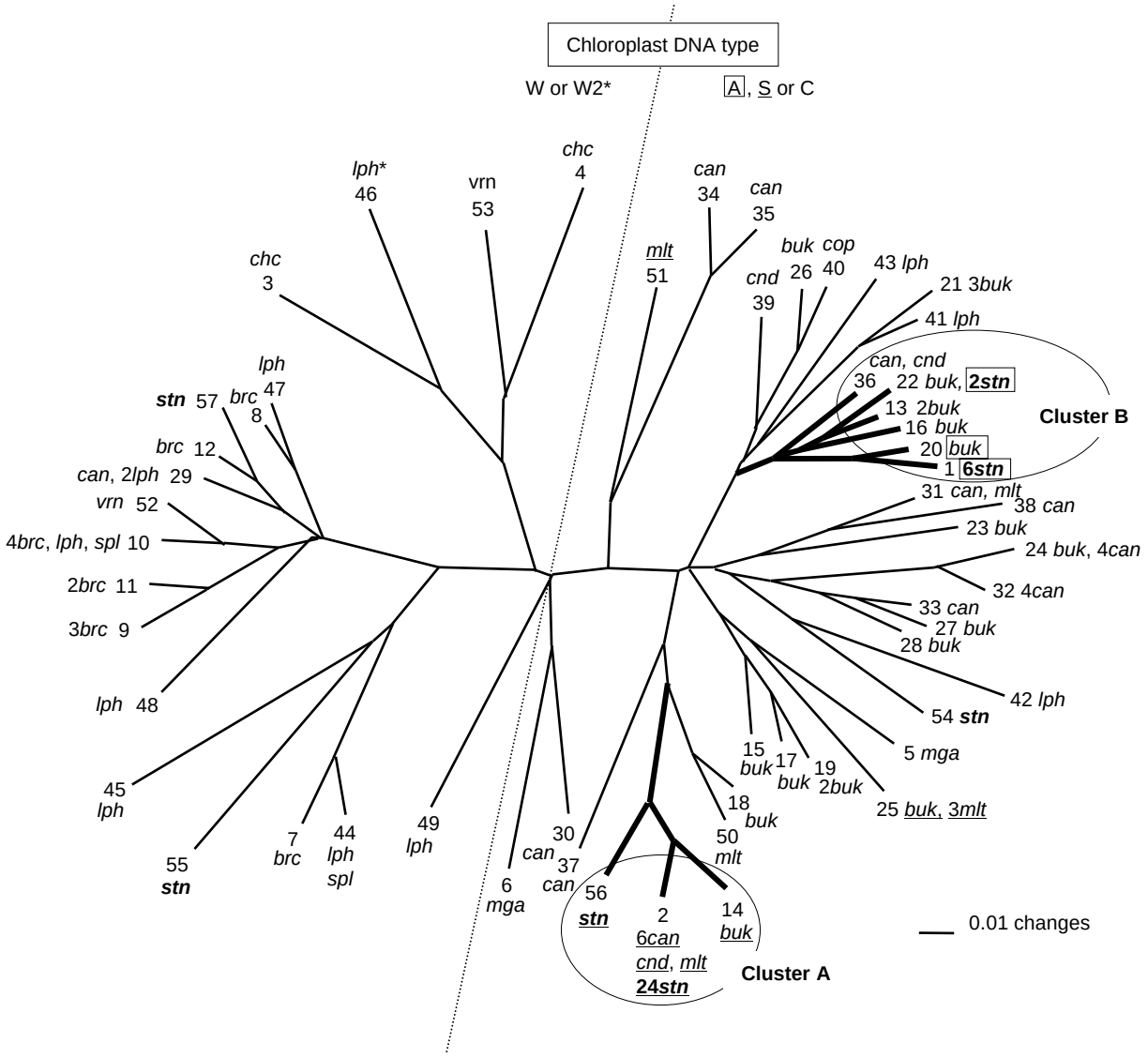
Taxon	No. of accessions	Microsatellites		CtDNA types		Haplotypes	
		No.	$H^{1)}$	No.	*h*	No.	*h*
S. stenotomum	36	25	0.31	4	0.46	7	0.52
S. brevicaule	12	11	0.21^{ns}	1	0	6	0.78
S. bukasovii	20	24	0.45^{ns}	3	0.27	16	0.93
S. canasense	23	29	0.53**	3	0.45	12	0.85
S. candolleanum	3	10	0.28^{ns}	2	0.31	3	0.67
S. leptophyes	12	32	0.53**	3	0.49	11	0.90
S. multidissectum	7	17	0.41^{ns}	2	0.41	6	0.82
'*S brevicaule* complex'$^{2)}$	78	40	0.60**	5	0.62	45	0.96

Wild species	86	44	0.62**	5	0.62	52	0.97

1) Difference in *H* between *S. stenotomum* and the other taxon was tested by *t* test. ns not significant, **significant at a 1% level.

2) '*S. brevicaule* complex' consisted of *S. brevicaule*, *S. bukasovii*, *S. canasense*, *S. candolleanum*, *S. coelestipetalum*, *S. leptophyes*, and *S. multidisecctum*.


Wild species
Peru
Bolivia
1 = buk
2 = can
3 = mlt
4 = lph
5 = cnd
6 = brc
7 = spl
8 = mga
9 = vrn
0 = cop
□ = Cluster A
○ = Cluster B
□ = haplotype 2
○ = A-type ctDNA
Solanum stenotomum
Peru
Bolivia


Chloroplast DNA type
W or W2*
A, S or C
chc 4
lph* 46
vrn 53
chc 3
can 34
can 35
mlt 51
buk cop 26 40
cnd 39
43 lph
21 3buk
41 lph
can, cnd
36 22 buk, 2stn
13 2buk
16 buk
20 buk
1 6stn
Cluster B
31 can, mlt
38 can
23 buk
24 buk, 4can
32 4can
33 can
27 buk
28 buk
42 lph
54 stn
5 mga
15 buk
17 buk
19 2buk
25 buk, 3mlt
18 buk
50 mlt
14 buk
2 6can cnd, mlt 24stn
56 stn
Cluster A
30 can
37 can
6 mga
49 lph
7 brc
44 lph spl
55 stn
45 lph
lph 48
3brc 9
2brc 11
4brc, lph, spl 10
vrn 52
can, 2lph 29
brc 12
stn 57
brc 47
lph 8
0.01 changes