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PHENOTYPIC AND GENETIC CHARACTERIZATION OF FOUR SPECIES OF THE *Solanum* genera, *Lycopersicon* SECTION

Caracterización fenotíca y genética de cuatro especies silvestres del género *Solanum*, sección *Lycopersicon*

María N. Morales Palacio[1]✉, Ángel R. Morales Astudillo[1], Adriana Artiles Valor[2], Yoamel Milián García[3] and Georgina Espinosa López[3]

ABSTRACT. The tomato (*Solanum lycopersicum* L.) is the most economically important vegetable worldwide and one of the most consumed vegetable in the world. It's wild relatives are native of various habitats ranging from Ecuador, Peru, and Chile and have been employed to generate varieties adapted to specific biotic and abiotic factors worldwide. In order to evaluate the morphological and genetic variation in the germplasm collection at the National University of Loja (UNL) in Ecuador, four wild species were selected: *Solanum pimpinellifolium*, *Solanum neorickii, Solanum habrochaites*, *Solanum lycopersicum* var. *cerasiforme*. With the morphological variables (20 quantitative, 20 qualitative) phenotypic differences in vegetative components and related to flower and fruit were detected. Only *S. habrochaites* was differentiated based on these variables. The diversity and genetic structure of the species were evaluated with 17 microsatellite loci. In spite of none of the variability indexes showed statistically significant differences due to the large variance presented, the species *S. neorickii* exhibited the lowest genetic variability values. The individual genetic distances, the number of groups genetically structured and the genetic differentiation (F_{ST}) were congruent and revealed four groups corresponding to each species tested.

Key words: germplasm bank, Ecuador, microsatellites, tomato, genetic variation

RESUMEN. El tomate (*Solanum lycopersicum* L.) es la hortaliza de mayor importancia económica en todo el planeta y uno de los vegetales más consumidos en el mundo. Las especies silvestres emparentadas son nativas de diversos hábitats que van desde Ecuador, Perú, hasta Chile y han sido empleadas para generar variedades adaptadas a factores bióticos y abióticos específicos en todo el mundo. Con el objetivo de evaluar la variación morfológica y genética en la colección de germoplasma de la Universidad Nacional de Loja (UNL) en Ecuador, se seleccionaron cuatro especies silvestres: *Solanum pimpinellifolium, Solanum neorickii, Solanum habrochaites, Solanum lycopersicum* var. *cerasiforme*. Con los caracteres morfológicos (20 cuantitativos, 20 cualitativos) se detectaron diferencias fenotípicas en los componentes vegetativos y los relacionados con la flor y el fruto. Sólo *S. habrochaites* se diferenció sobre la base de estos caracteres. La diversidad y estructura genética de las especies se determinó con 17 *loci* microsatélites. A pesar de que ninguno de los índices de variabilidad mostró diferencias estadísticamente significativas, debido a la gran varianza que presentan, la especie *S. neorickii* mostró los menores valores de variabilidad genética. Las distancias individuales, el número de grupos estructurados genéticamente y la diferenciación genética (F_{ST}) fueron congruentes y revelaron cuatro grupos diferenciados correspondientes a cada especie estudiada.

Palabras clave: banco de germoplasma, Ecuador, microsatélites, tomate, variación genética

[1] IDENTIGEN. Laboratorio Biomolecular, Consorcio Torre 1, Azuay y Sucre, Loja, Ecuador.

[2] Centro de Investigaciones Pesqueras, 5ta y 246. Barlovento, Playa. CP 19100,Ciudad de la Habana, Cuba. Afiliación actual Miami Dade College, USA.

[3] Facultad de Biología, Universidad de la Habana, Ciudad de la Habana, Cuba.

✉ nataliamorales88@yahoo.es

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is considered one of the most important vegetables in many countries of the world and accounts for approximately 30 % of their consumption in

developing countries, representing near 65 % of worldwide production (1).

The primary center of its origin and greater distribution of wild relative species comprises the regions located along Andean mountains, within the territory now shared by Colombia, Ecuador, Peru, Bolivia and Chile, including Galapagos Islands (2), where wild *Solanum* species grow spontaneously. These plants can live in a variety of habitats, from sea level in the arid Pacific coast up to about 3300 m over sea level in many valleys on the west side of the Andes (3).

The earliest cultivated tomato is the botanical variety *Solanum lycopersicum* var. *cerasiforme* (cherry tomato), which lives in the tropical and subtropical regions of Ecuador and Peru (4), from where it was probably widespread to all tropical America in pre-Columbian times. In Ecuador, *S. neorickii* and *S.habrochaites* are also found as wild species, while *S. chilense*, *S. chmielewskii*, *S. pennellii*, *S. peruvianum* in Peru and *S. chilense* in northern Chile, all of them with green fruits.

The most advanced edible forms, *S. cheesmaniae* and *S. pimpinellifolium*, with red or yellow fruits are wildly found at maturity stage in Ecuador, whereas the latter one in Peru.

Wild tomato species have individual attributes of potential significance for breeding cultivated varieties (5); however, before promoting the use of wild species, it is necessary to know the genetic variability preserved in their natural habitat by making an inventory, description and characterization.

A large number of accessions of wild tomato species and cultivated varieties are kept in genebanks all over the world. Morphological characters enable to differentiate accessions, determine promising materials and its usefulness for genetic breeding. Moreover, molecular markers such as microsatellites (SSR) are advantageous to differentiate accessions, identify the variety and trace the product, as they have proved to be highly powerful to distinguish closely related tomato cultivars(6-8), without being affected by environmental conditions. Techniques of morphological and molecular characterization complement each other, in such a way that molecular diversity does not consider genotype-environment interactions; therefore, both methods together can provide a broad and real knowledge of species diversity.

The genebank from the Biotechnology Center at the National University of Loja-Ecuador (UNL) has about 2,000 *Solanum* accessions. This material has been partially characterized at a morphological level; besides that Ecuadorian accessions have been traditionally sub-represented in previous works. This study was aimed at phenotypically and genetically characterizing four wild *Solanum* species mostly collected in Ecuadorian territory, in order to determine the extension of their variability and establish the usefulness of molecular markers to differentiate the proposed species.

MATERIALS AND METHODS

The material used in this study belongs to the genebank collection from the Biotechnology Center at UNL, which was gathered in a wide geographical area of the primary center of tomato diversity (Ecuador and Peru, Figure 1) by the biotechnology team in partnership with Galapagos National Park, the Agrodiversity Preservation and Breeding Center of Valencia (COMAV) from the Polytechnic University of Valencia, the National University of Piura-Peru and “Pedro Ruiz Gallo” National University of Lambayeque-Peru.

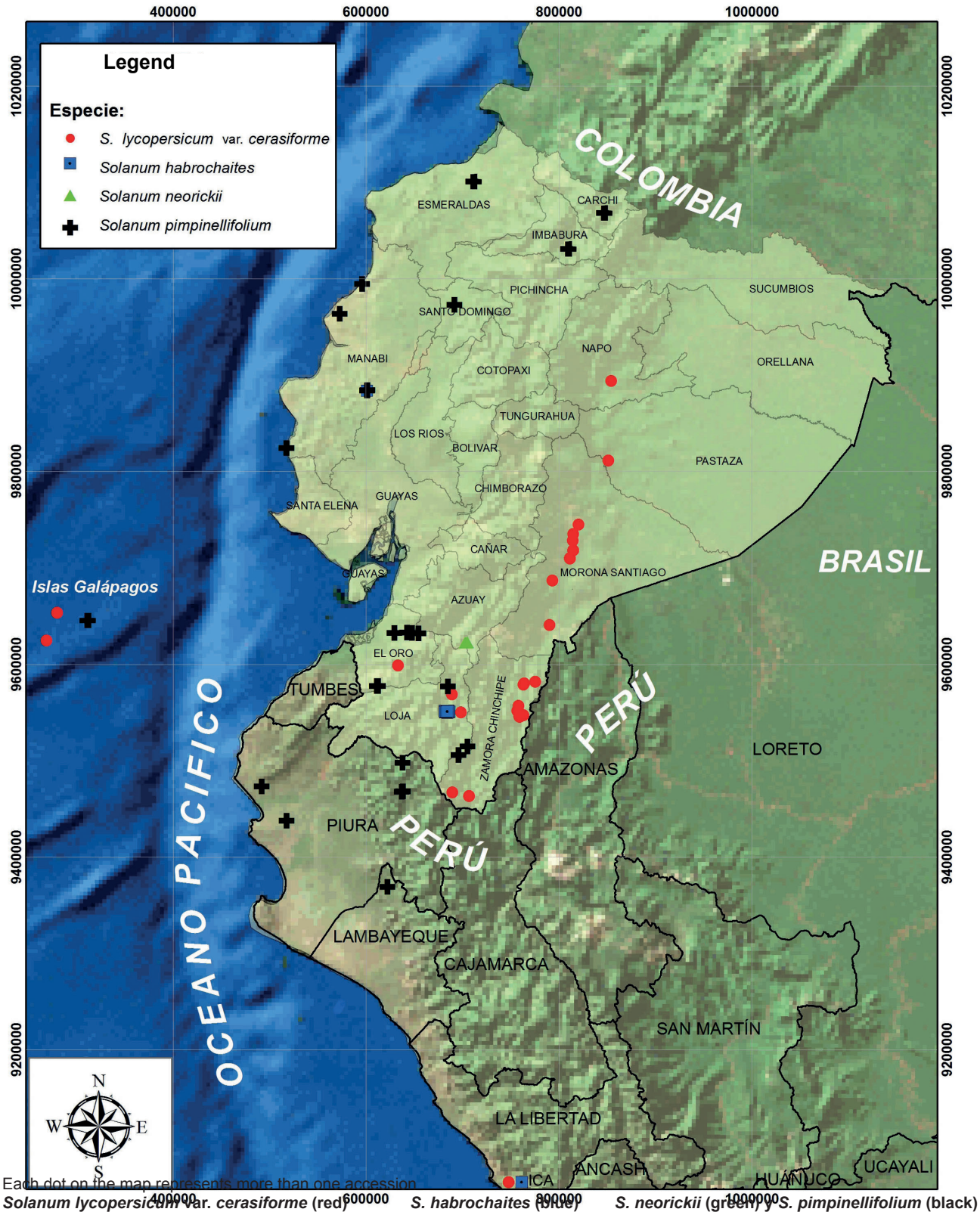
Geographical coordinates were determined through a global positioning system, based on the relative location with respect to an artificial satellite system (GPS, Magellan XL, San Dimas-California).

Four wild species of *Solanum* genus *Lycopersicon* section [two from *Lycopersicon* group (*Solanum pimpinellifolium* L., *Solanum lycopersicum* var. *cerasiforme* Dunal), one from *Eriopersicon* group (*Solanum habrochaites* S. Knapp and D.M. Spooner) and one from *Arcanum* group (*Solanum neorickii* D.M. Spooner, G.J. Anderson and R.K. Jansen).

The morphological characterization was performed in 659 plants from 146 accessions (average of five plants per accession) of the above mentioned four wild species (9), while a molecular characterization was estimated in 194 plants, as detailed in Table I.

The analysis of morphological variables is described by several authors (9). In this study, uncorrelated variables (≤0,85) were separated in two groups: the vegetative one and the other related to flower and fruit components. A classification analysis of main coordinates was performed with these groups and the four wild species by using Gower similarity index, through PAST statistical package (10).

Euclidean (quantitative) and similarity (qualitative) distance matrix was obtained with these variables, with the purpose of building a



Each dot on the map represents more than one accession

Solanum lycopersicum var. *cerasiforme* (red) *S. habrochaites* (blue) *S. neorickii* (green) y *S. pimpinellifolium* (black)

Figure 1. Collecting sites of wild species of *Solanum* genus *Lycopersicon* section

Table I. Number of accessions and plants per accession used in the study

Species	Morphological characterization		Molecular characterization	
	Accession number	Plant number	Accession number	Plant number
S. lycopersicum var. *cerasiforme*	57	228	57	61
S. habrochaites	36	208	36	61
S. pimpinellifolium	40	147	40	48
S. neorickii	13	76	13	24

Values are listed for each of the four wild species considered

cladogram through UPGMA clustering method. Subsequently, to check construction strength, a Mantel test was performed by NTSYSpc-2,02 g program (11).

For a molecular characterization, DNA was extracted from 100 mg leaves preserved at -86 °C, using cetyl-trimethyl ammonium bromide (CTAB) method with some modifications.

DNA quality and quantity was extracted and estimated by agarose gel electrophoresis at 0,8 % and ethidium bromide (3 %), run for 50 minutes at 140 volts in a TBE 1X buffer. Bands were visualized on an UVITEC BTS-20 LM at 320 nm trans-illuminator and its intensity was compared with the band pattern of *Trackit Lambda DNA/Hind III fragment* (INVITROGEN) marker. Then, DNA was taken to a final concentration of 10 ng/uL for a final volume of 50 uL in a Tris EDTA buffer solution.

In addition, 27 microsatellite primers were chosen out of a total of 44 pairs described (12), based on their polymorphism and band quality assessment (Table II).

Each amplifying reaction was performed for a final volume of 15 uL: 1 uL genomic DNA, 1,5 uL 10X buffer [1X], 1,5 uL of each primer [1 uM], 1 uL deoxy-ribonucleotides [0,1 mM] (biosynthesis), 0,5 units of polymerase DNA *Taq* enzyme (INVITROGEN) and $MgCl_2$ up to a concentration of 2 mM, except some *loci* where the final salt concentration was changed (Table II). PCR was made in a BioRad iCycler thermocycler with the amplifying program described (12): one cycle of94 °C (3 min), 30 cycles as follows [55° (45 sec),72° (1 min, 45 sec), 94 °C (45 sec)]. A final extension cycle at 55 °C (45 sec) and 72 °C (3 min). Hybridization temperature and the number of cycles varied depending on the primers employed (Table II).

Amplified products were separated by vertical electrophoresis in PAGE denaturing gels, run at constant 75 W, 45 to 50 °C, for 60 to 90 minutes, depending on the expected fragment size. To assign allele size (pb), a 30-330 pb *AFLP® DNA Ladder* (Life Technologies) size marker was used.

GenAlEx 6.1 program was employed to estimate mean values of genetic diversity parameters (13), taking into account sample size. FSTAT program version 2.9.3 (14) was used to calculate diversity indexes, such as allelic richness (RA), observed heterozygosity (Ho), intraspecific diversity (Hs), total diversity (Ht), Nei interspecific variation coefficient (Dst), Weir and Cockerham F_{ST} stadigraph and statistical differences between species by means of Bonferroni correction. Species linkage unbalance was performed with 10,880 permutations and a probability value fitted to 5 % of 0,000092.

Individual genetic distances were estimated to construct exploratory trees through Neighbor-Joining clustering algorithm with Population (15) and MEGA 5 (16) programs.

Structure 2.2 program (17) was also used to infer genetic structure, which ran under the following conditions: 500 000 iterations during pre-simulation period and 1 000 000 repetitions of Montecarlo and Markov chains (MCMC); seven *k* values were calculated and cluster number (*k*) was estimated as the maximized number to Δ*k* parameter with Structure Harvester (18). The method employs a model with *k* populations (*demos*) that are assumed to be in Hardy-Weinberg (HW) and gametic balance, although the first premise was not attained.

Table II. Microsatellite loci employed and PCR conditions applied for amplifying in species of *Solanum* genus *Lycopersicon* section

No.	*Locus name*	Repetition	Fragment size	Primer sequence (direct, reverse)	PCR conditions		
					T (°C) Hibridation	Cycle no.	$MgCl_2$ (mM)
1	LE20592	$(TAT)_{15-1}(TGT)_4$	166 pb	5' -CTGTTTACTTCAAGAAGGCTG-3'	55	30	2
				5' -ACTTTAACTTTATTATTGCCACG-3'			
2	LE21085	$(TA)_2(TAT)_{9-1}$	104 pb	5' -CATTTTATCATTTATTTGTGTCTTG-3'	50	30	2
				5' -ACAAAAAAAGGTGACGATACA-3'			
3	LE2A11	$(ATCT)_{5-1}$	157 pb	5' -AATTTTGTAAGGAGAAGACGG-3'	55	30	2
				5' -TCATATTCTTCACACCAAAGG-3'			
4	LEATPACAa	$(TA)_7$	189 pb	5' -TTACTTACTCCCCTCCAACTC-3'	50	30	2
				5' -CGTTTGGTTACAAGAGAATTG-3'			
5	LEATPACAb	$(GA)_7$	184 pb	5' -GTATGTCAAATCTCTCTTGCG-3'	55	30	2
				5' -ACTCTCCATCGTCTCTTTCAC-3'			
6	LECAB9	$(TA)_6(CA)_3$	118 pb	5' -TTTATTATCCCAGAAGCCTTC-3'	50	30	2
				5' -CCTCACATTTAAACAAATTGC-3'			
7	LECHI3	$(TA)_{6-1}(GA)_4$	128 pb	5' -TAACAATCAAAAGAACTTCGC-3'	55	30	2
				5' -ATCCCCTTATTGATTACATCC-3'			
8	LECHSOD	$(CTT)_6$	195 pb	5' -TTATCAATTCATCATTGTGGC-3'	55	30	2
				5' -AGGGGTAGTGACAGCATAAAG-3'			
9	LEDIH4RE	$(AAT)_5(AAG)_2$	90 pb	5' -TTTTGTAATCATCTTGGAAAC-3'	50	30	2,5
				5' -ATTGTGTTATGATGATATTTG-3'			
10	LEE8	$(TA)_6$	152 pb	5' -TCTTTAGTAGCTCAGTGGCAG-3'	55	30	2
				5' -GGCCAACTAAATCGTTTATTC-3'			
11	LEEF1Aa	$(TA)_8(ATA)_9$	131 pb	5' -AAATAATTAGCTTGCCAATTG-3'	55	30	2
				5' -CTGAAAGCAGCAACAGTATTT-3'			
12	LEEF1Ab	$(TTA)_{4-1}A(TTA)A(TTA)_4$	245 pb	5' -AATTTAACAATTGCCAAGTGA-3'	55	30	2
				5' -TGGCTGAAGAATTTTAAATGA3'			
13	LEGAST1	$(TA)_{12}$, $(TG)_{8-2}$	143 pb	5' -ATCTCTATTGTTTTCGACTCG-3'	55	30	2
				5' -TCTGTTGTTGCTGCTGCTC-3'			
14	LEHMG2A	$(AAC)_5$	254 pb	5' -ATCTGAAGAGCCTGTTTATCC-3'	55	30	2
				5' -AAAGCGTAACGACATGTAAAG-3'			
15	LEILV1B	$(T)_8(TA)_{10}(T)_5$	143 pb	5' -GATCGACACATTTGAATTTGT-3'	50	30	2
				5' -GGTCACTAATTAATTGATTCC-3'			
16	LELAT59G	$(TA)_{9-1,}$ $(TA)_{11}$	168 pb	5' -AAAAGGGGTATGAACATTAGG-3'	55	30	2,5
				5' -GCATCTATCGTCTTGTCACTC-3'			
17	LELEUZIP	$(AAG)_{6-1}TT$ $(GAT)_7$	105 pb	5' -GGTGATAATTTGGGAGGTTAC-3'	55	30	1,5
				5' -CGTAACAGGATGTGCTATAGG-3'			
18	LEMDDNa	$(TA)_9$	211 pb	5' -ATTCAAGGAACTTTTAGCTCC-3'	55	30	2
				5' -TGCATTAAGGTTCATAAATGA-3'			
19	LEMDDNb	$(TG)_4(TA)_4$	280 pb	5' -TAAATACAAAAGCAGGAGTCG-3'	55	30	2
				5' -GAGTTGACAGATCCTTCAATG-3'			
20	LENIA	$(TA)_{6,}$ $(TG)_5$	210 pb	5' -TTAAGATTGTATTCATCATGG-3'	55	30	2
				5' -CTTTAGGCTTGTAATGGAGTG-3'			
21	LERBCS3B	$(TG)_{6-1}(TA)_{8-1}$	198 pb	5' -AAACCTTGACATTACCTCCAT-3'	55	30	2
				5' -AGGAAGGTACGACAGAGTCTC-3'			

22	LESODB	$(TTC)_6$	207 pb	5' -TTATCAATTCATCATTGTGGC-3'	55	30	2
				5' -AGTAAGGGGTTTAGGGGTAGT-3'			
23	LESSF	$(CCCCA)_4$	216 pb	5' -TACGCTCTCAAGTACCGTAAG-3'	55	30	2,5
				5' -CCTACATTGACATGACCAAAT-3'			
24	LESSRPSPGb	$(C)_{16}$	332 pb	5' -AACATTAGTTTGATTGGATGG-3'	50	35	2
				5' -TTAAACTTTGCTTGACTTTCC-3'			
25	LEWIPIG	$(CT)_{8\text{-}1}(AT)_4$	254 pb	5' -GAGTCAAAGTTTGCTCACATC-3'	55	30	2
				5' -CTCTTCTGAACTTGCTTTGAG-3'			
26	LPHFS24	$(TA)_6$	149 pb	5' -TTGGATTTACAAGTTCGATGT-3'	55	30	2
				5' -GCATTTGACTTGATAGCAGTC-3'			
27	LEACC2G	$(AAAT)_3$	147 pb	5-TTCCCAGGAAAGTAATTATCC-3	50	30	2
				5-GTTCAAGCTAGAAGCTACACG-3			

RESULTS AND DISCUSSION

This work included the morphological and molecular study of four wild *Solanum* species collected from its natural habitat in Ecuador. The species have been traditionally sub-represented in previous studies (19) and have shown differences in relation to the accessions collected in Peru (20, 21). Wild cherry tomato (*S. lycopersicum*) accessions (21) have also been characterized to select genotypes based on agronomically interesting characters for cultivated tomato breeding.

Moreover, genes controlling fruit shape in *S. lycopersicum* (22) have been recently cloned. In the latest two years, with the application of a new generation sequencing, new markers of single nucleotide polymorphisms (SNPs) are referred, that will be useful to characterize the genetic diversity and structure of wild species, as well as the search for *loci* linked to quantitative traits (23, 24).

Morphological characterization

Figure 2 shows results of the main coordinate analysis, considering uncorrelated morphological variables in two groups (vegetative and those related to flower and fruit). *S. habrochaites* species is the only one that makes a well-differentiated conglomerate and *S. neorickii* differs from *S. habrochaites*. *Cerasiforme* variety appears in all groups, but it is not differentiated by the characters used.

The cluster analyses performed (9), considering all uncorrelated morphological variables together, were consistent with those of the present work, as clusters of species with little differentiation were formed, except *S. habrochaites*. The qualitative variable analysis behaved similarly.

Molecular characterization

The molecular characterization using 27 microsatellite *loci* showed that 10 out of them did not amplify with any or some species. LE21085 and LEGAST1 primers did not amplify with *S. lycopersicum* var. *cerasiforme*; LEATPACAa, LEILV1B and LENIA with *S. habrochaites*; LESSF with *S. pimpinellifolium*; LE20592 and LELAT59G with *S. neorickii* and LEACC2G with no species; while LESSRPSPGb only amplified with *S. pimpinellifolium*, so it is considered a potential diagnosis for the species.

The molecular characterization using 17 microsatellite *loci* that amplified with four species provided information on the genetic diversity and structure of wild species studied.

These 17 *loci* were found in a linkage unbalance for all samples and each species separately, which implies that each *locus* gives independent information to determine the genetic structure of species. However, most of the species for these *loci* showed a probability value near the limit, compared to the lack of Hardy-Weinberg genetic balance.

This result is explained by the fact that each accession was collected at distant sites (several meters up to tens of kilometers) along Ecuadorian and Peruvian territories; therefore, a whole group of them does not make up a population. Other authors recorded the lack of genetic balance in some microsatellite *loci* of *Solanum lycopersicoides* and *S. sitiens* species, who studied their genetic diversity and structure (25).

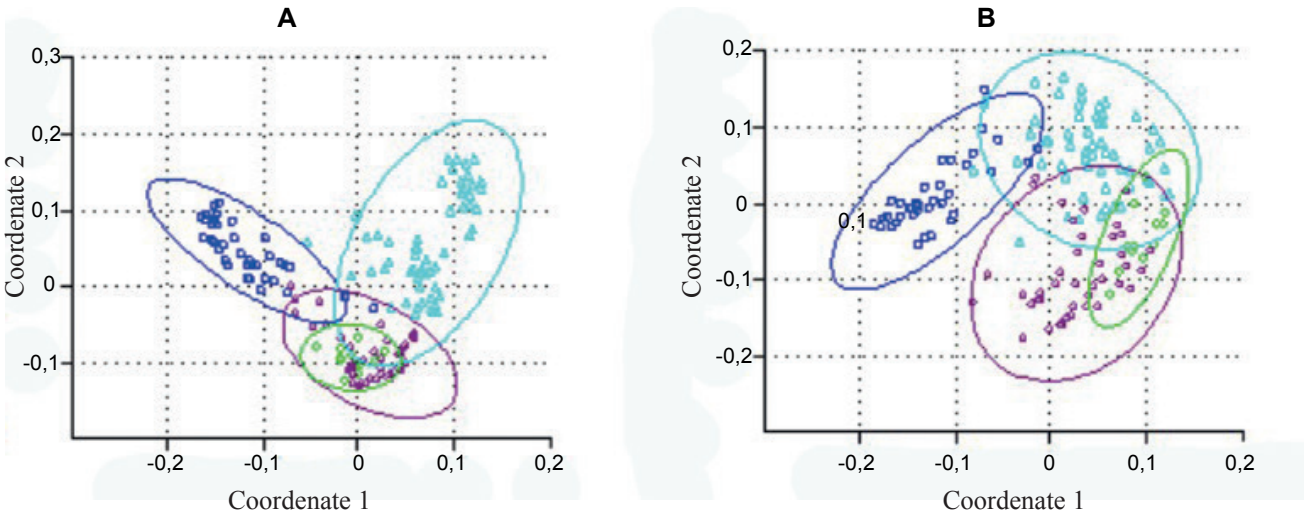
Likewise, they found high coefficients of consanguinity and considered the existence of Walhund effects as a result of so large geographic areas occupied by clusters formed.

The mean values of genetic variability parameters for 17 *loci* analyzed are shown in Table III. Although none of the indexes showed significant differences, from the statistical point of view, due to the large variance they present, *S. neorickii* species showed the lowest values.

The fact that *S. neorickii* shows lower values at the genetic variability indexes is explained because this species is limited in Ecuador within a small geographical area under the same climatic conditions, which means lower diversity. *S. pimpinellifolium* with a smaller sample size (22 %), compared to *S. lycopersicum* var. *cerasiforme* presents similar genetic variability values. Other studies refer to *S. pimpinellifolium* as the greatest genetic variability species (20).

This species is widely and freely distributed along the roadsides from Ecuador to Peru, which has also allowed obtaining a large species collection. It is not easy to find *S. lycopersicum* var. *cerasiforme*, since it is restricted to Amazonian natives' house gardens. In addition, allogamy is difficult due to the inserted stigma of most of its flowers, which would confirm a lower genetic variation of this species, as it was obtained in the present work.

Since the collection of *S. habrochaites* used in this study comes from the greatest concentration area of this species (Loja, southern Ecuador), there were high genetic variability values, which together with a greater variability verifies the fact that populations of this species abound on riverbanks, fallow lands, hillsides, gardens, among others.



Quantitative morphological data of 146 accessions belonging to four wild species of Solanum genus Lycopersicon section: S. lycopersicum var. cerasiforme (turquoise), S. habrochaites (blue), S. neorickii (green) and S. pimpinellifolium (purple)
(A) Data related to vegetative characters (B) Data related to flower and fruit components

Figure 2. Main Coordinate Analysis through Gower similarity index

Table III. Mean values of genetic variability parameters in four wild species of *Solanum* genus *Lycopersicon* section, estimated at 17 microsatellite loci by GenAlEx statistical program

Population	*S. lycopersicum var. cerasiforme*	*S. pimpinellifolium*	*S. habrochaites*	*S. neorickii*
N	61	48	61	24
Na	3,412±0,310	3,647±0,284	3,765±0,466	2,765±0,349
Ne	2,418±0,245	2,738±0,187	2,722±0,336	2,188±0,247
RA	3,205±0,305	3,538±0,268	3,592±0,431	2,722±0,338
NAP	1,176±0,356	1,353±0,383	1,059±0,315	0,765±0,250
He	0,506±0,058	0,614±0,029	0,53± 0,062	0,459±0,062

The mean and its standard error are shown
(N) Individual number (Na) Mean allelic number per locus (Ne) Effective allelic number
(RA) Allelic richness (NAP) Private allelic number (He) Expected heterocigosity

On the other hand, the self-incompatibility condition that forces *S. habrochaites* to cross-pollination with neighboring individuals of the same species can justify its lower variability compared to *S. pimpinellifolium*. The above mentioned information is based on other authors' findings (19), who reported that heterozygosities observed in a population of western Loja-Ecuador were lower than the ones expected if the population would be in Hardy-Weinberg balance, which means that there is certain degree of selfing or crosses between sister plants in all populations.

The individual distance tree (Figure 3A) appeared through Cavalli-Sforza genetic distances of genotyped individuals. Four groups with well-marked boundaries among species are observed in this representation: *S. pimpinellifolium*, *S. habrochaites*, *S. neorickii* and *S. lycopersicum* var. *cerasiforme.*

The structure generated with information of these 17 *loci*, using Structure program, displays a *k* value of four as the maximized number to Δ*k* parameter (Figure 3B).

The clusters formed by Structure program (Figure 4B) show that *S. lycopersicum* var. *cerasiforme* (N=61) have lower allocation coefficients than 2 %, both of *S. pimpinellifolium*, *S. neorickii* and *S. habrochaites*.

S. pimpinellifolium (N=48) belongs to another cluster, where some individuals show allocation percentages of *S. neorickii* (between 1 and 5 %) and most of them show allocation percentages of *S. lycopersicum* var. *cerasiforme* (between 1 and 4 %). *S. neorickii* (N=24) has a small allocation coefficient (1 %) of *S. lycopersicum* var. *cerasiforme* and *S. habrochaites*, while *S. habrochaites* (N=61) presents the highest allocation percentages of *S. neorickii* (between 5 and 20 %) and of some individuals of *S. lycopersicum* var. *cerasiforme* and *S. pimpinellifolium* (1 %). F_{ST} values showed total congruence with the differentiation of all species described.

Due to low levels of allogamy of *S. lycopersicum* var. *cerasiforme*, it is unlikely to be a gene flow between this species and *S. pimpinellifolium* (20). Also, a study conducted with AFLP markers between these two species refers that they are genetically distinct and there is little gene flow between them (3). However, *S. lycopersicum* var. *cerasiforme* presents both plants with flowers and inserted stigma as well as exerted stigma whereas *S. pimpinellifolium* has always exerted stigmas, which could favor interspecific crossing and allogamy between these species.

Results from phenotypic and genetic characterization of the four wild species proposed are shown in Figure 4. Their fruits have morphological distinctions that allow differentiating them (Figure 4A); however, the fruit is not always available.

The genetic structure of wild species (Figure 4B) shows *S. habrochaites* and *S. neorickii* are differentiated in an individual cluster, so that each species results from populations with poor redifferentiation over time.

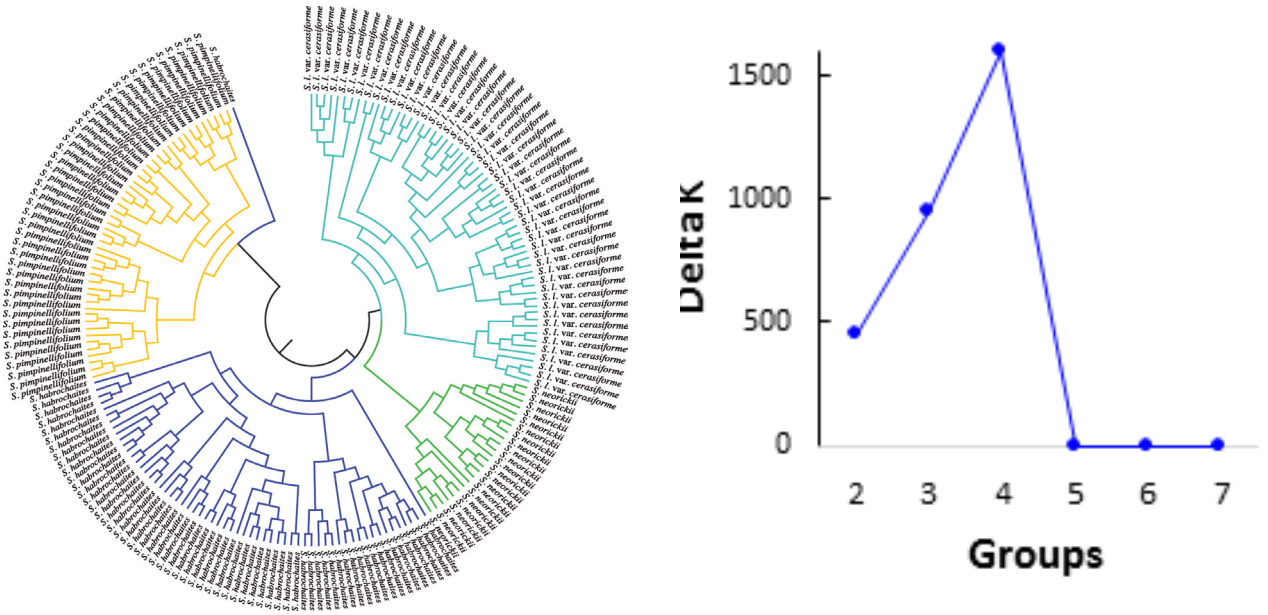
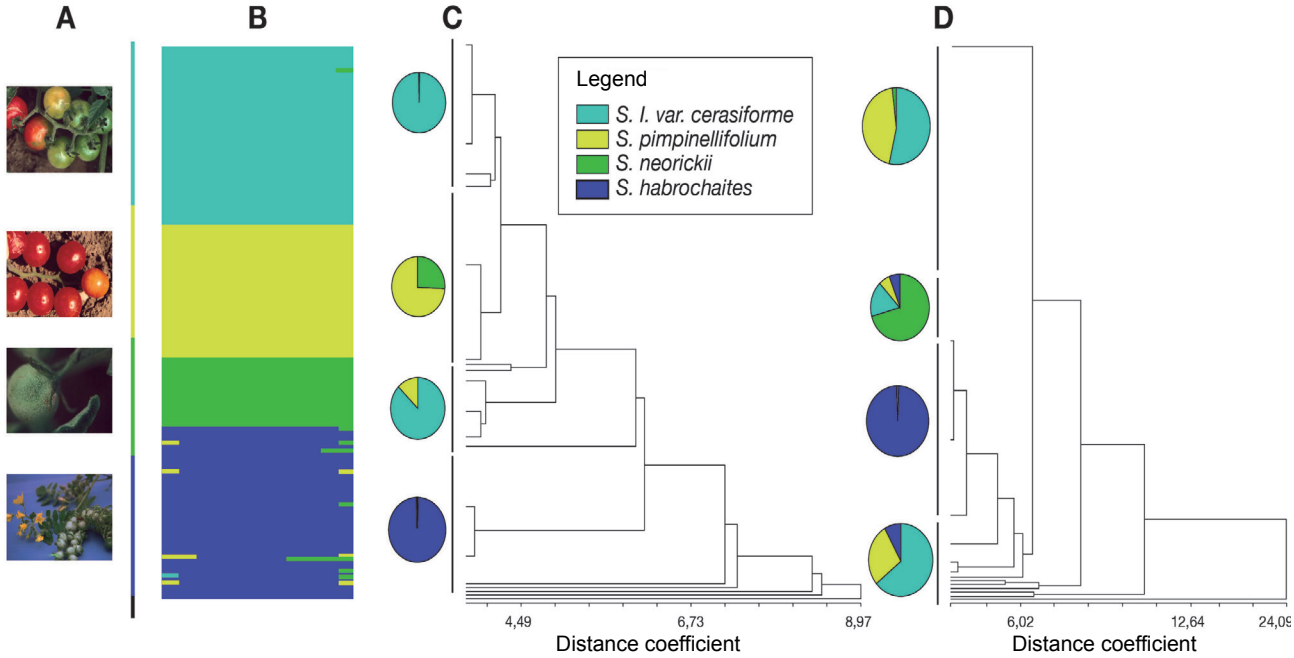


Figure 3. (A) Exploratory tree of Cavalli-Sforza individual genetic distances obtained by Population program and displayed by MEGA program. The four wild species of *Solanum* genus *Lycopersicon* section are shown: *S. pimpinellifolium* (yellow), *S. habrochaites* (blue), *S. neorickii* (green), *S. lycopersicum* var. cerasiforme (turquoise). (B) Estimated number of groups (k) for all four species



(A) Images of wild species fruits, a representative variation is observed in relation to the species to which they belong.
(B) Genetic structure obtained by Structure program from SSR molecular markers, in which each individual is represented by a horizontal bar that highlights each group formed.
(C) Distance cladogram based on uncorrelated quantitative morphological descriptors (≤0,85) using Euclidean distance coefficient and Pearson correlation coefficient (r=0.80060).
(D) Similarity cladogram constructed with qualitative data by UPGMA clustering method.
Figures C and D show phenetic relationships between species by circles divided into colors.
The plot indicates when species appear in more than one group.

Figure 4. Morphological and molecular analysis of four wild species of *Solanum* genus *Lycopersicon* section collected in Ecuador, Galapagos Islands and Peru

The cladogram obtained through arbitrary node cuttings by means of uncorrelated quantitative variables (Figure 4C) shows *S. habrochaites* in a defined cluster separated from the remaining species. *S. pimpinellifolium* and *S. neorickii* are mixed and separated in another one. Concerning *S. lycopersicum* var. *cerasiforme*, although most individuals appear in a defined cluster, others are next to *S. pimpinellifolium*.

The cladogram built with qualitative variables grouped by UPGMA method (Figure 4D) also separates *S. habrochaites* from the other species; nevertheless, the remaining ones appear mixed along the cladogram. The fact that *S. pimpinellifolium* is joined to *S. lycopersicum* var. *cerasiforme* is mainly explained because both have edible red fruits.

CONCLUSIONS

♦ Morphological variables are generally useful to recognize wild species from *Solanum* genus *lycopersicon* section characterized in this study; however, they allow discriminating *Solanum habrochaites* species effectively.

♦ Microsatellite *loci* used show a similar genetic variability among species, except *S. neorickii* with a lower genetic variation. The genetic structure obtained between species indicates they are well differentiated.

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