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REGULAR ARTICLE

Morphological variation of wild peppers (*Capsicum* spp.) from the state of Tabasco, Mexico

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ABSTRACT

The pepper (*Capsicum* spp.) is one of the most important vegetables in Mexico. The aim of this study was to collect wild peppers and characterize *in situ* their morphological diversity. From January to November 2015, field trips were made to 54 locations in 15 municipalities in the state of Tabasco, Mexico; 131 collections were obtained in which a total of 23 plant, flower and fruit variables were evaluated. With the mean values of the variables, principal component (PC) and cluster analyses were performed. The first three PCs explained 65.2% of total morphological variability, with the variables fruit length, fruit shape, fruit width, leaf width, leaf length, plant height and branch density providing a greater explanation for the diversity. Cluster analysis grouped the 131 collections into two groups, one formed by the morphotypes 'Amashito', 'Garbanzo' and 'Ojo de cangrejo', with characteristics of *C. annuum* var. Glabriusculum, and the second group with the morphotype 'Pico de paloma' with characteristics of the species *C. frutescens* L. It is concluded that the wild peppers of the State of Tabasco have morphological diversity, which must be preserved as a genetic resource of interest.

Keywords: Cluster analysis; Genetic resources; Morphological diversity; Morphotypes; Principal component analysis

INTRODUCTION

The pepper (*Capsicum* spp.) is one of the most important vegetables in Mexico and the world. The genus *Capsicum* is composed of about 37 species, of which only *Capsicum annuum* L, *C. chínense* Jacq., *C. frutescens* L., *C. baccatum* L. and *C. pubescens* Ruíz & Pav. were domesticated (Bosland and Votava, 2012; Zhang et al., 2016). The species *C. annuum* L., which stands out in economic importance and cultivated area, has its center of diversity and domestication in Mexico (Hayano-Kanashiro et al., 2016).

In Mexico, wild peppers of the species *C. annuum, C. frutescens*, *C. ciliatum* and *C. lanceolatum* (Castañón-Nájera et al., 2008; Vera-Guzmán et al., 2011) can be found from sea level to over 2000 meters above sea level (Hernández-Verdugo et al., 2012; Kraft et al., 2013). Studies on wild peppers indicate high levels of morphological variation between and within species and populations (Hernández-Verdugo et al., 2015; Hayano-Kanashiro et al., 2016), with variation in characters related to phenology, plant, flower and fruit shape (Bosland and Votava, 2012). The most important for morphological description are those that have less environmental influence, such as fruit, leaves and stem (Castañón-Nájera et al., 2008), which can be used to satisfactorily discriminate between species of wild peppers (Hernández-Verdugo et al., 2012).

In southeastern Mexico and in particular in the state of Tabasco, wild pepper populations of the species *C. annuum* var. *glabriusculum* and *C. frutescens*, which show morphological and genetic variability, can be found (Castañón-Nájera et al., 2008). This is a valuable resource that is at risk of being lost due to adverse factors such as habitat destruction, hurricanes, floods and droughts (Narez-Jiménez et al., 2014). Therefore, it is necessary to collect and characterize the diversity of wild peppers in order to know their characteristics and to conserve this resource for the breeding of cultivated peppers (Hayano-Kanashiro et al., 2016). Due to the above, the aim

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of this work was to characterize *in situ* the morphological diversity of the different wild pepper morphotypes found in the state of Tabasco, Mexico.

MATERIALS AND METHODS

The work was carried out in the state of Tabasco, located between 18º 39' 03" and 17º 15' 03" NL, and 90º 59' 15" and 94º 07' 48" WL, on the coastal plain of the Gulf of Mexico, with an area of 24738 km^2. The predominant climate types in the state are warm humid with abundant rainfall throughout the year (*Af*) in 19.64% of the area, warm humid with abundant rainfall in summer (*Am*) in 75.97% of the area and warm sub-humid with rainfall in summer A(*w*) in 4.39% of the area. Annual precipitation is between 2500 and 3000 mm and the average annual temperature is 26.6 ºC (INEGI, 2015).

The exploration sites were selected based on studies carried out by Castañón-Nájera et al. (2008) and Narez-Jiménez et al. (2014), and information on collection dates and sites provided by the wild pepper sellers in the markets of the explored municipalities. The collections were carried out from January to November 2015 through walks along paths and visits to orchards, paddocks, and banana and cocoa ecosystems in 54 localities in the municipalities of Balancán, Cárdenas, Centla, Centro, Comalcalco, Cunduacán, Emiliano Zapata, Huimanguillo, Jalapa, Jalpa de Méndez, Macuspana, Nacajuca, Tacotalpa, Teapa and Tenosique in the state of Tabasco, Mexico.

In the plants or groups of plants collected, 23 morphological plant, flower and fruit variables were evaluated (Table 1) on the basis of the descriptors for *Capsicum* proposed by IPGRI-CATIE-AVRDC, (1995) and the Technical guide for varietal description of pepper (*Capsicum annuum*) of SAGARPA-SNICS, (2014). Based on fruit and flower variables, the species to which the collections belong was identified (Andrews, 1995; Hernández-Verdugo et al., 1999; Bosland and Votava, 2012). *In-situ* morphological characterization was performed for 131 plant collections or groups of plants, at elevations from sea level up to 495 meters above sea level. For each collection, coordinates were recorded with GPS (Garmin eTrex®) to locate the collection sites (Fig. 1). From each plant or group of plants, all the ripe fruits were taken, stored in paper bags and transferred to the Agricultural Sciences Research Center at the Juárez Autonomous University of Tabasco (*Universidad Juárez Autónoma de Tabasco*), where they were left to dry at room temperature, after which the number of seeds per fruit was counted.

With the means of each variable, a first principal component analysis (PCA) was carried out; it included the 131 collections and the 23 variables, which were standardized to $\mu = 0$ and $\sigma^2 = 1$. This first analysis allowed determining the 16 most important variables for describing the variability of the collections, with which a second PCA was carried out. In both analyzes, PC estimation was performed with the correlation matrix, so that the variables involved in the analysis were equally important (Johnson and Wichern, 2007). The significance of the eigenvalues and eigenvectors was determined by Keizer's rule (1960). After a cluster analysis was performed from the squared Euclidean distance matrix with Ward's minimum variance method, the cut height was decided based on the cubic clustering criterion and the pseudo-t^2 and pseudo-F statistics (Johnson and Wichern, 2007). All statistical analyses were performed using the SAS 9.4 program (SAS, 2013).

RESULTS AND DISCUSSION

A total of 131 wild pepper collections were obtained from the morphotypes 'Amashito', 'Ojo de cangrejo', 'Garbanzo' and 'Pico de paloma' (Fig. 2). The collections of the morphotypes 'Pico de paloma' (45.8%) and 'Garbanzo' (30.5%) account for a higher proportion of the total, while the morphotypes 'Amashito' (18.3%) and 'Ojo de cangrejo' (5.4%) are found to a lesser extent. The lower proportion of 'Ojo de cangrejo' and 'Amashito' is due to the fact that they are the wild peppers preferred for local consumption and sale (Narez-Jiménez et al., 2014).

The first principal component analysis (PCA) with the 23 variables showed that the first three PCs explained 45.1% of total morphological variability. In this regard, Castañón-Nájera et al. (2008) indicate that by eliminating the variables that contribute little or nothing to the explanation of PC1, PCA is improved. By performing PCA with the 16 selected variables, the first three PCs explained 65.2 % of total variability (Table 2), with the first five PCs having significant values according to Keiser (1960).

PC1 explained 35.8% of total morphological variation, which was positively determined by the variables fruit shape, fruit shape at the juncture with the pedicel and fruit apex shape, and negatively by the variables fruit length and fruit weight. This indicates that the component was determined by fruit variables, which is consistent with previous studies on the morphological diversity of wild peppers, in which it was reported that the first component was explained by fruit variables (Narez-Jiménez et al*.,* 2014). PC2 explained 17.1% of total morphological variability, with the variables stem diameter, plant height, plant width, leaf color, leaf length, leaf width, fruit length, fruit width and number of seeds per fruit being the variables that contributed most to

Table 1: Plant, flower and fruit descriptors

	Variable	Measurement method
Plant	1) Stem diameter	In millimeters (mm) with a digital Vernier caliper graduated in mm.
	2) Plant height	In centimeters (cm) with a measuring tape graduated in cm.
	3) Plant width	In centimeters (cm) with a measuring tape graduated in cm.
	4) Stem pubescence	3=sparse, 5=intermediate and 7=dense.
	5) Plant growth habit	3=prostrate, 5=intermediate, 7=erect and 9=other (specify).
	6) Branch density	3=sparse, 5=intermediate, 7=dense.
	7) Leaf color	1=yellow 2=light green, 3=green, 4=dark green.
	8) Leaf shape	1=deltoid, 2=oval, 3=lanceolate.
	9) Leaf length	In millimeters (mm) with a digital Vernier caliper graduated in mm.
	10 Leaf width	In millimeters (mm) with a digital Vernier caliper graduated in mm.
Flower	11) Number of flowers per axil	1=one, 2=two, 3=three or more, 4=many flowers, but each in an individual axil, 5=other.
	12) Flower position	3=slope, 5=intermediate, 7=erect.
	13) Corolla color	1=white, 2=yellow-lemon, 2=yellow-pale orange, 4=yellow-greenish, 5=purple with white base, 6=orange, 7=purple with light purple base, 8=purple, 9=other.
	14) Corolla shape	1=round, 2=campanulate and 3=other (indicate).
Fruit	15) Color of fruit in mature state	1=white, 2=yellow-lemon, 3=yellow-pale orange, 4=yellow-orange, 5=pale orange, 6=orange, 7=light red, 8=red, 9=dark red, 10=purple, 11=brown, 12=black, 13=other.
	16) Fruit length	In millimeters (mm) with a digital Vernier caliper graduated in mm.
	17) Fruit width	In millimeters (mm) with a digital Vernier caliper graduated in mm.
	18) Fruit weight	In grams (gr), with a digital scale.
	19) Fruit pedicel length	In millimeters (mm) with a digital Vernier caliper graduated in mm.
	20) Fruit shape	1=elongated, 2=almost round, 3=triangular, 4=bell-shaped, 5=bell-shaped and block, 6=other (indicate).
	21) Fruit shape at junction with the pedicel	1=acute, 2=obtuse, 3=truncated, 4=chordate, 5=lobed.
	22) Fruit apex shape	1=pointy, 2=blunt, 3=sunken, 4=sunken and pointy, 5=other.
	23) Number of seeds per fruit	Count.

the explanation of the component. PC3 explained 12.3% of total morphological variability, being determined in greater proportion by the variables stem diameter, plant height, plant width and branch density, whereas PC4 explained 9.5% of total morphological variability, and was explained by the variables branch density, fruit width, fruit weight and number of seeds per fruit. Regarding PC5, it explained 7.5% of total variability, being determined by the variables stem diameter, plant growth habit and fruit width. The results indicate that all plant structures showed variability, which is useful for collection discrimination. In this regard, Castañón-Najera et al. (2008) indicate that the morphological variability in wild peppers is explained to a greater degree by fruit and plant variables.

The distribution of the wild pepper morphotypes by means of the first two principal components, PC1 and PC2 (Fig. 3), gave rise to two groups, the first with the morphotypes 'Amashito', 'Garbanzo' and 'Ojo de cangrejo', while the second group was formed with the morphotype 'Pico de paloma'. The collections of the first group were located in the positive quadrant of PC1 and the positive and negative quadrants of PC2 (Fig. 3). The first group was formed by 71 collections that have fruits of lower weight, a round shape and a blunt or sunken apex, while the second group was formed by 60 collections, which are located in the negative quadrant of PC1 and are distributed in the positive and negative quadrant of PC2; this group was formed by collections that have fruits of greater weight, an elongated shape and a pointy fruit apex. In this regard, Castañón-Nájera et al. (2008) and Narez-Jiménez et al. (2014) report that the state of Tabasco has wild pepper morphotypes characterized by round and pointy fruits.

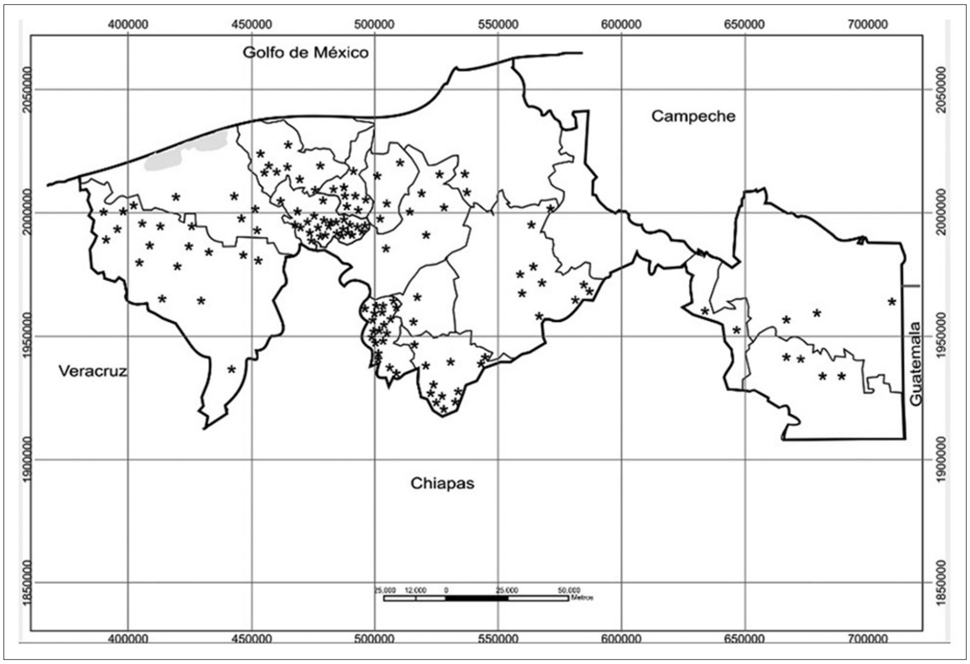

Fig 1. Geographic location of the localities where the 131 collections of wild peppers were made in the state of Tabasco, Mexico.

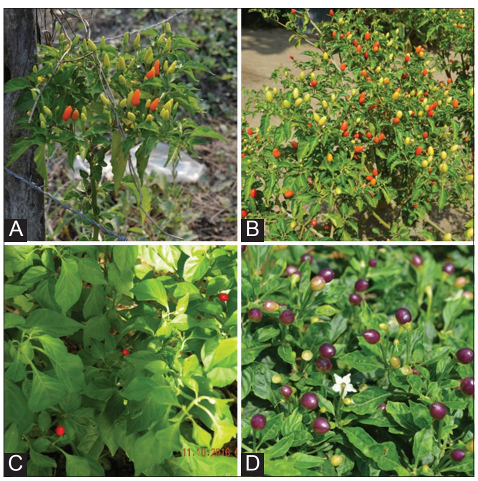

Fig 2. Wild pepper morphotypes. A = 'Pico de paloma', B = 'Garbanzo', C = 'Amashito' and D = 'Ojo de cangrejo'.

The fruits of the morphotypes 'Amashito', 'Garbanzo' and 'Ojo de cangrejo' are mainly characterized by having round-shaped fruits, a characteristics that according to Andrews (1995), Mogkolporn and Taylor (2011), Bosland and Votava (2012), Kraft et al. (2013) and Hayano-Kanashiro et al. (2016) is one of the main traits presented by the species *Capsicum annuum* var. *glabriusculum*, while the morphotype 'Pico de paloma' has elongated, pointy-shaped fruits, a characteristic that according to Andrews (1995), Hernández-Verdugo et al. (1999) and Kraft et al. (2013) is shared by the wild fruits of the species *Capsicum frutescens* L. On this, Hernández-Verdugo et al., (1999), Castañón-Nájera et al. (2008) and Narez-Jiménez et al. (2014) reported the presence in the state of Tabasco of wild populations of the species *C. annuum* var. *glabriusculum* and *C. frutescens* L., with morphological and genetic variability.

Cluster analysis enabled grouping the 131 collections into two morphologically different groups (Fig. 4), by taking as reference a semi-partial R^2 distance of 0.10 units. Group 1 was formed by collections of the morphotypes 'Amashito', 'Ojo de cangrejo' and 'Garbanzo', which by their flower and fruit characteristics belong to the species *C. annuum* var. *glabriusculum*, which is the wild form of the cultivated pepper *C. annuum* var *annuum* L. (Andrews, 1995; Hernández-Verdugo et al*.,* 1999). This group is characterized by having plants with an average height of 1.13 m, fruit weight of 0.30 g, fruit length of 9.2 mm, fruit width of 6.8 mm and 16 seeds per fruit, values that are within the ranges reported for the species by Hernández-Verdugo et al. (1999) and Narez-Jiménez et al. (2014). As for group 2, it was formed by 60 collections of the morphotype 'Pico de paloma', which belongs to *C. frutescens* L., with average fruit weight of 0.40 g, length of 16.2 mm, width of 5.7 mm, 17 seeds per fruit and average plant height of 1.18 m, values within the range reported for wild fruits of *C. frutescens* L. (Hernández-Verdugo et al*.,* 1999; Castañón-Nájera et al*.,* 2008).

CONCLUSIONS

The morphological variability found in the 131 collections of wild peppers from the state of Tabasco is grouped into the species *C. annuum* var. *glabriusculum* ('Amashito', 'Garbanzo' and 'Ojo de cangrejo') and *C. frutescens* L. ('Pico

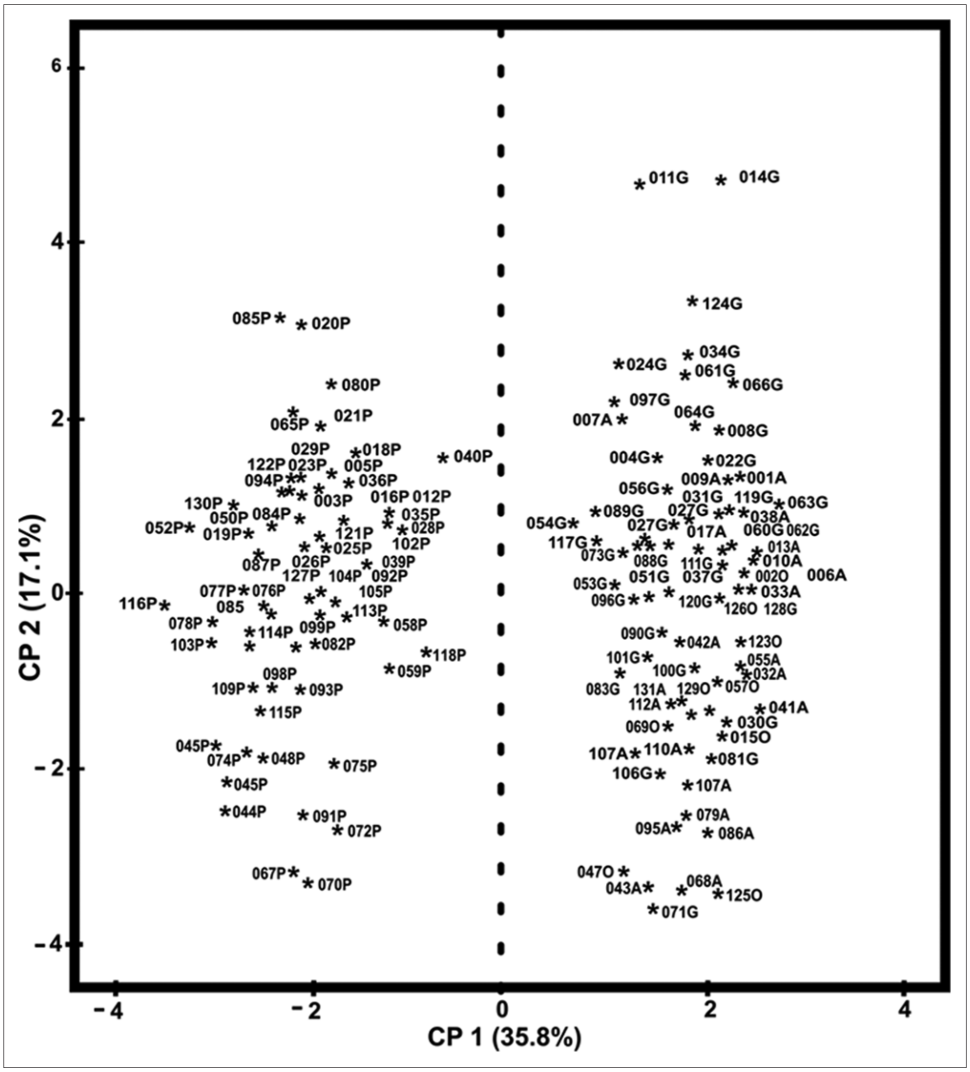

Fig 3. Distribution of the 131 collections of *Capsicum* spp., as a function of PC1 and PC2 obtained with the correlation matrix. P=pico paloma, G=garbanzo, A=amashito and O= ojo de cangrejo.

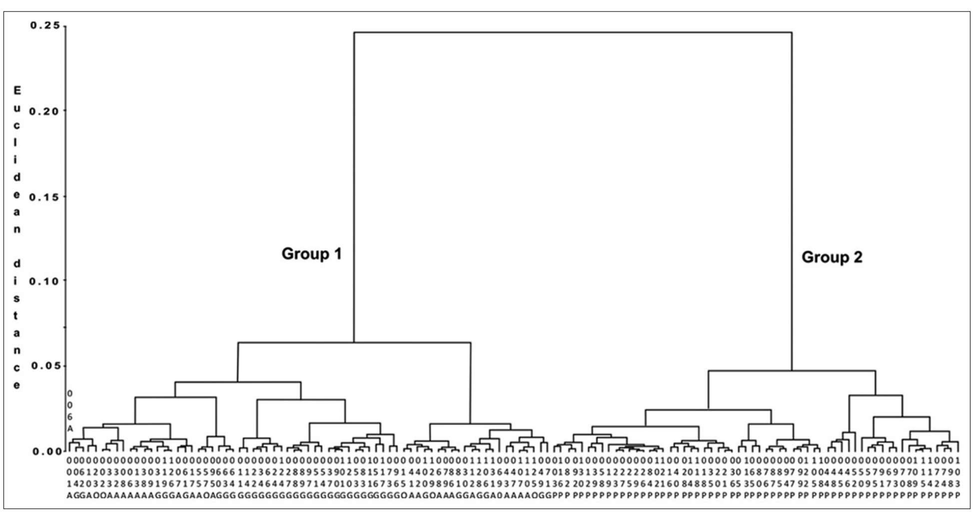

Fig 4. Ward's clustering of 131 pepper (*C. annuum* var. *glabriusculum* and *C. frutescens*) collections where A = 'Amashito', O = 'Ojo de cangrejo', G = 'Garbanzo' and P = 'Pico de paloma'. Group 1 = *C. annuum* var. *glabriusculum*, Group 2 = *C. frutescens*.

Table 2: Eigenvectors and eigenvalues of the PCs with variables of the highest descriptive value

Variable	PC1	PC2	PC3	PC4	PC5
Stem diameter	0.107	0.285*	0.337*	−0.252*	0.219*
Plant height	–0.050	0.219*	0.448*	−0.175	0.042
Plant width	0.007	0.217*	0.526*	−0.210*	−0.048
Plant growth habit	0.001	0.055	−0.135	−0.175	0.755*
Branch density	−0.076	−0.104	0.493*	0.205*	−0.050
Leaf color	0.173	0.252*	−0.098	−0.220*	−0.358*
Leaf length	0.047	0.492*	−0.219*	−0.158	−0.100
Leaf width	0.052	0.476*	−0.244*	−0.106	0.010
Color of fruit in mature state	−0.025	0.166	0.030	0.162	−0.399*
Fruit length	−0.410*	0.202*	−0.029	0.064	0.011
Fruit width	0.171	0.351*	−0.053	0.421*	0.204*
Fruit weight	−0.223*	0.172	0.127	0.476*	0.183
Fruit shape	0.472*	−0.023	0.057	0.156	0.025
Fruit shape at junction with the pedicel	0.477*	−0.012	0.062	0.146	0.026
Fruit apex shape	0.477*	−0.012	0.062	0.146	0.026
Number of seeds per fruit	−0.137	0.238*	−0.002	0.452*	−0.043
Eigenvalue	4.123	2.731	1.964	1.526	1.193
Variation explained	0.358	0.171	0.123	0.095	0.075
Cumulative variation	0.358	0.529	0.652	0.747	0.822

*Significant according to Keiser (1960).

de paloma'), indicating that these peppers constitute a genetic resource that must be conserved as a reservoir of genes with the potential to solve agricultural problems. The species *C. annuum* var. *glabriusculum* and *C. frutescens* L. are present in all the municipalities included in the study, with the morphotypes 'Pico de paloma' and 'Garbanzo' being found to a greater extent than the morphotypes 'Amashito' and 'Ojo de cangrejo'.

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Authors' contributions

J.C.V.V.: carried out the fieldwork, data collection and the first draft of the paper; C.M.Q.: participated in the development of the research and revised the article; E.C.L.: participated in the development of the research, data analysis, and the writing and revision of the paper; R.O.O.: participated in the development of the research and in the revision of the article; P. P. R.: participated in the data analysis and discussion.

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