

Morphovariability of capulín (*Prunus serotina* Ehrh.) in the central-western region of Mexico from a plant genetic resources perspective

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Abstract The capulín, or black cherry tree (*Prunus serotina* Ehrh.) is a tree with edible fruits native to North America that has been used not just as a source of food but also as a source of energy (its wood) since prehispanic times. Mexico has three of the five botanical subspecies in the *P. serotina* botanical complex, which have not yet been characterized for agricultural purposes. It is desirable to determine whether capulín trees comprise a homogeneous group or whether there are separate morpho-types. This study focuses on the morphological variability of seven populations from the western and central regions of Mexico. We used the Hill & Smith Analysis and graphical methods to analyze and interpret data with respect to 22 quantitative morphological characters and 17 qualitative morphological

characters, all from different plant organs. Two major geographically and morphologically related groups were clearly identified, and the morphological variability in the study zone was related to taxonomic, genetic and agronomic factors. The results are discussed from a genetic resources perspective. The genetic, cultural, ecological and agronomic implications of these results are also considered, as well as the potential uses for the species. From these results, an agro-morphological characterization can be developed, in order to identify interesting types of capulín for fruit and seed production in agro-industry, or as rootstock for related species and forestry uses. Our results have implications for the conservation and sustainable use of capulín genetic resources in the central and western regions of Mexico.

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Introduction

The Capulín (*Prunus serotina* Ehrh.) is a tree with edible fruits species that grows in temperate zones, from southern Canada to southern Bolivia, and in altitudes between 1000 and 3200 meters in Mexico

(Niembro 1990). It is a species that has been known and used in Mexico since prehispanic times. The actual importance of this species is a result of its consumption in central Mexico (Muratalla 1984). The fruit of the tree is a type of cherry, often called capulín, capuli or capolin in Mexico (Popenoe and Pachano 1922). Agronomic studies regarding to productivity and phenology of capulín (Avitia and Muratalla 1982; Muratalla 1984; Buendía 1988; Hernández and Muratalla 1990) have been directed by the Muratalla group in Mexico.

In 2003, a study of the agro-morphological diversity of the capulín was performed in Michoacán, Mexico (Agustín et al. 2003). In that study, agronomic variables were evaluated for trees under different conditions of *in situ* vegetation management: tolerated¹ trees and wild trees. The tolerated trees could be considered as semi-cultivated plants because these trees were not planted by the farmers, but since they represented a source of fruits and wood, the farmers then decided to conserve them. Casas et al. (2007) have proposed the term “let standing” to denominate such plants. They defined this term as a plant-human interaction which “includes practices directed to maintain within human-made environments useful plants that occurred in those areas before the environments were transformed by humans.” For simplicity, we decided to use the term “tolerated” as a synonym for the term “let standing”.

The zone in which Agustín et al. performed their study contains the Purepecha ethnic group. Several trees grow in their agricultural fields; additionally, wild capulín can be found in the forest adjacent to those fields. Agustín and collaborators conclude that variables such as fruit weight and yield are not static through time, and studies to identify useful descriptors for this plant species are needed.

In the United States, *P. serotina* has been studied for forestry purposes because of its capacity to regenerate in perturbed environments (Buendía 1988 and Maynard et al. 1991). The existing studies reporting on *in vitro* regeneration (Maynard et al.

1991 and Espinosa et al. 2006) have focused on genetic transformation and reproductive sterility induction (Xiaomei and Pijut 2008).

One molecular diversity study of samples from the United States, Mexico and Ecuador identified micro-satellite markers specific to *P. serotina* (Downey and Iezzoni 2000); however, the taxonomic identities of the plants used were not taken into account in the study. With respect to the botanical relationships with other *Prunus* species, Aradhya et al. (2004) performed molecular characterization and found that *P. serotina* is related to *Prunus mahaleb* L., an important species in rootstock generation. The use of *P. serotina* for food stands out among its potential uses, since its fruit and seeds are more nutritious than those of the sweet cherry or sour cherry tree, according to Avendaño (2000). In Mexico, the fruit of capulín is used for making food or liquors, the wood is used in furniture manufacture because of its hardness and durability, and the leaves and flowers have been noted to have therapeutic properties for respiratory diseases (Losoya 1982).

Important studies by Popenoe and Pachano (1922) in South America, McVaugh (1951) in North America, and Rzendowski and Calderón de Rzendowski (2005) in Mexico have described the diversity of capulín, with the intention of assigning botanical identities to each species. *P. serotina* is composed of five botanical subspecies (McVaugh 1951). In Mexico, three subspecies are present: *P. serotina* ssp. *capuli* (Cav.) McVaugh, *P. serotina* ssp. *serotina* (Ehrh.) McVaugh and *P. serotina* ssp. *virens* (Wooton et Standl.) McVaugh. The botanical systematics of the subspecies take into account primarily the morphological characteristics of the leaves, flowers and fruit. The botanical classification is ambiguous for some subspecies. For example, in the *capuli* and *serotina* subspecies, morphological differences with respect to the size and shape of the leaves are minimal. Some other intra-species differences are based on the agricultural handling of the tree. The subspecies *capuli* is described as cultivated, and the subspecies *serotina* is considered to be in the process of being domesticated (Avendaño 2000). The fruit size, seed size, length of flower branch and number of flowers on each branch are the main morphological characteristics that are used in determining domestication. Although these systematic elements are used in distinguishing the capulín subspecies, identification of intra-specific variation requires very specific examination.

¹ Plants or trees that have existed prior to when the farmer began to work the land. Trees in agricultural fields or parcels, where the farmer has permitted their development for any reason. Now, the farmer uses these trees, but he does not perform agricultural maintenance (e.g. pruning or fertilization) on these trees (Casas et al. 1997).

The capulín is a fruit-bearing tree distributed widely throughout the central and western regions of Mexico (central-western region). It is necessary to create a program to exploit this tree, starting from an accurate characterization of its different potential uses. Basically, it is necessary to determine whether the capulín trees in central Mexico comprise a homogeneous group, or whether there are geographically separated morpho-types. The present study is a description of the morphological variability of *P. serotina* Ehrh. in the central-western region of Mexico. It serves to delineate approaches for the management of the genetic resources of the species and to enable an agromorphological characterization for the identification of promising varieties of the capulín for fruit production (fresh fruit, rootstocks) and other uses.

Materials and methods

Plant material collection

Plant specimens from seven tree populations of *P. serotina* Ehrh. from four states of Mexico, Michoacán, Querétaro, México State and Tlaxcala (Table 1), were collected for the herbarium. The number of sampled trees was directly proportional to the size of the tree population. The dispersion and reproduction characteristics of the capulín populations, as well as their agricultural handling, suggest a count of 20 individuals per 10,000 m² in the central and western regions of Mexico. For the wild populations, 25% of the trees in a 10,000 m² area were sampled (*Mich1* = 6, *Mich2* = 5, *Mich3* = 4, *Qro* = 6, *Tlax1* = 5 and *Tlax2* = 5 individuals). Of the

Table 1 Origin, environment and taxonomic identities reported for seven *P. serotina* Ehrh. populations sampled in the Central-Western region of Mexico

Label (Population)	Municipality and Mexican State	Environment		Taxonomic identity reported
		Geographic location and elevation	Climate type ^a	
<i>Tlax1</i>	Españita, Tlaxcala	19°29'19" N 98°26'20" W 2720 m	C (w ₂) (w) b (e)g Ruderal in corn fields	<i>Prunus serotina</i> ssp. <i>capuli</i> ^b
<i>Tlax2</i>	Nanacamilpa, Tlaxcala	19°31'17" N 98°30'32" W 2650 m	C (w ₂) (w) b (e)g Ruderal in corn fields with maguey	<i>Prunus serotina</i> ssp. <i>capuli</i> ^b
<i>Mich1</i>	Pátzcuaro, Michoacán	19°30'16" N 101°38'32" W 2400 m	Cb (w ₂) (w) (e)g Coniferous forest margins	<i>Prunus serotina</i> ssp. <i>serotina</i> ^c
<i>Mich2</i>	Pátzcuaro, Michoacán	19°30'39" N 101°38'30" W 2400 m	Cb (w ₂) (w) (e)g Coniferous forest margins	<i>Prunus serotina</i> ssp. <i>serotina</i> ^c
<i>Mich3</i>	Erongarícuaro, Michoacán	19°36'00" N 101°43'00" W 2100 m	C (w ₂)(w) b (e)g Agricultural fields, rockground, and coniferous forest margins	<i>Prunus serotina</i> ssp. <i>serotina</i> ^c and <i>Prunus</i> <i>serotina</i> ssp. <i>virens</i> ^c
<i>Qro</i>	Pinal de Amoles, Querétaro	21°08'12" N 99°37'32" W 2360 m	C (w ₂) w'' b (i')g Community orchard, coniferous forest hill margins	<i>Prunus serotina</i> ssp. <i>serotina</i> ^c
<i>Mex</i>	Chapingo, México	19°29'58" N 98°52'44" W 2270 m	C (w ₀) w b (i')g Collection in experimental fruit orchard	<i>Prunus serotina</i> ssp. <i>capuli</i> ^b

^a According to Köppen classification adapted to Mexico (García 1988)

^b Taxonomic identity reported by McVaugh (McVaugh 1951)

^c Taxonomic identity reported by Rzendowski and Calderón de Rzendowski (2005)

samples collected from the capulín collection of Chapingo Autonomous University, ten individuals, with desirable agronomic characteristics, such as fruit size (diameter ≥ 2 cm.) and color, a sweet flavor, and a productivity percentage greater than 50% (flowers/fruits), were recognized. These features were taken into account during the analysis.

Methods

The likely distribution of the species in Mexico was estimated with the use of information from 295 *P. serotina* subspecies accessions from the herbarium databases and botanical reports analyzed in the Geographical Information System (GIS) FloraMap ver. 1.02 (Arnold 2000). The western and central regions of Mexico were selected as the study regions, based on the GIS results. The determination of the collection sites was aided by botanical reports and communication with Rzendowski and Calderón de Rzendowski (2005). For the climate data and formulas, the Köppen Climate Classification, as modified by García, was used (García 1988).

The descriptor list was developed from the works of McVaugh (1951), Avendaño (2000), Rzendowski and Calderón de Rzendowski (2005) and the Protocol of Distinctness, Uniformity, and Stability Test of *Prunus avium* L. Sweet Cherry (CPVO 2006). The complete list of capulín descriptors contains 54 general characters (morphological, cytogenetic and agricultural management-related). However, for the present study, only 39 were used. These descriptors are the morphological characters that can be measured in the field and easily evaluated with software analysis. These 39 morphological characters include 22 quantitative variables and 17 qualitative variables (Table 2). These characters were used to evaluate 41 herbarium samples of the capulín from seven western and the central Mexico populations. A descriptive analysis and multivariate methods were used in the data analyses. The flower filaments were pressed, dissected, photographed and measured with the aid of UTHSCSA ImageTool 3.0[®] Software (UTHSCSA 1998).

For each tree, three herbarium samples were taken from three different zones in the middle part of the treetop. Each sample included a reproductive branch with a minimum of three floral branches. The branches without fruit were collected during a later

visit. Around 200 fruits were collected and photographed. Such images were analyzed for color determination and as a source of morphological data (diameter, roundness, size) through image analysis. Trunk color was determined at the collection site with the use of the Munsell Plant Tissue Color Chart (Munsell Color Company 1997), at a point one meter from the ground. Finally, 41 herbarium specimens (with three replicates) were collected, and their descriptor values and passport data were obtained using the mean values of three repetitions for each one. Each collected herbarium sample was labeled, pressed and mounted as a herbarium plant specimen to verify its taxonomic identity.

The Hill and Smith analysis was used for the statistical analysis (Hill and Smith 1976). With this method, simultaneous analysis of the quantitative and qualitative multi-state data was possible. The statistical language R ver. 2.5.1 (R Development Core Team 2007) for Mac OS 10.4.9 and the library ADE4 version 1.4.3 for R (Thioulouse et al. 1996) were used to obtain dual diagrams (Dray and Dufour 2007). The 3D scatterplot was visualized with Normal Contour Ellipsoids in the JMP software (SAS Institute 2007). To determine the variables involved in differentiating the populations, an ANOVA, the Wilcoxon-Kruskal-Wallis test and the HSD Tukey test were used.

Results

Potential distribution and sampling results

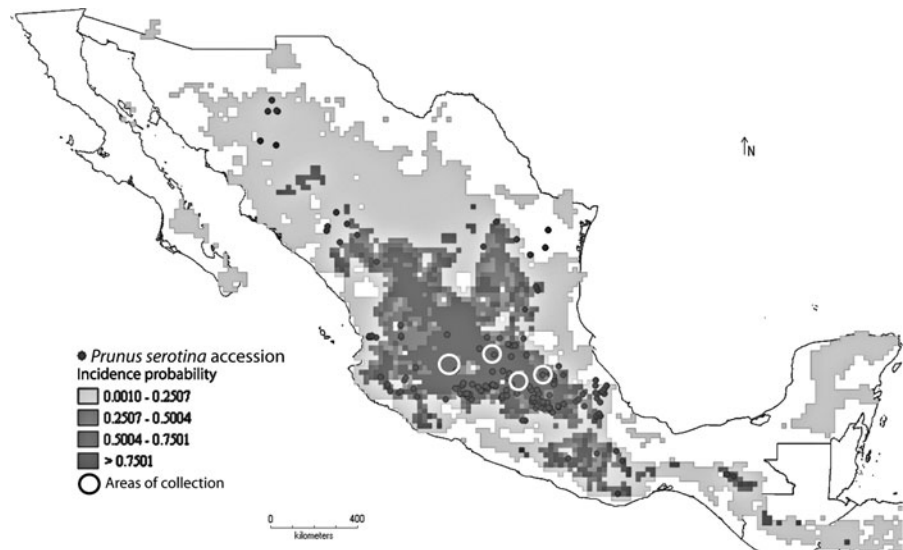
The map generated by FloraMap (Fig. 1) shows that *P. serotina* is distributed in four main regions in Mexico: northern, western, central and south-eastern, with the central and western regions having the greater incidence of the species. In addition, the three subspecies present in Mexico share ecological niches in the western and central regions, which includes the states of Michoacán, Mexico State, Tlaxcala and Querétaro.

The capulín in the west and center of Mexico grow in areas near the *Quercus* and *Pinus* forests with 400–900 mm of rainfall per year. The species prefers acidic volcanic soils. The estimated distribution corresponds to the ecological niches described by Venero (1966) and Argueta et al. (1994). McVaugh (1951) recognized the central and western regions as

Table 2 Morphological descriptors utilized for morphological variability evaluation of seven *P. serotina* Ehrh. populations in the Central-Western region of Mexico

Organ	Descriptor number	Morphological descriptor	Label	Measure unity
Complete tree	1	Productivity percentage	POP	Quantitative–Qualitative
	2	Tree form	TRF	Qualitative
	3	Flowering to pruning time	FPT	Qualitative
	4	Flowering season	FWS	Qualitative
	5	High tree	HGT	Qualitative
Stems and branches	6	Trunk color	TKC	Qualitative-Munsell scale
	7	Internode length in young steams	ILS	cm
	8	Thickness of young stems	TYS	cm
Leaves	9	Second basal leaf of flowering branch length	2BL	cm
	10	Second basal leaf of flowering branch thickness	2BT	cm
	11	Length/thickness quotient length of second basal leaf of flowering branch	LT2	NA
	12	Petiole length of second basal leaf of flowering branch	PL2	cm
	13	Teeth quantity in second basal leaf margin of flowering branch per 1 cm ²	TQM	Number · cm ⁻²
	14	Leaf upside green color intensity	LGI	Qualitative-Munsell scale
	15	L2H/LP2 Quotient	LPQ	NA
Flower	16	Nectars color	NLC	Qualitative-Munsell scale
	17	Young leaves color	YLC	Qualitative-Munsell scale
	18	Flower branch length	FBL	cm
	19	Flower pedicel length	FPL	cm
	20	Stamen length	STL	cm
	21	Flower diameter	FLD	cm
	22	Number of flowers per branch	NFB	No
Fruit	23	Fruit pedicel length	FPL	cm
	24	Fruit diameter	FRD	cm
	25	Fruit form	FRF	Qualitative
	26	Fruit basal form	FBF	Qualitative
	27	Thickness of fruit pedicel	TFP	cm
	28	Fruit abscission point presence	FAP	Yes/no
	29	Fruit color	FRC	Qualitative-Munsell scale
	30	Thickness of fruit skin	TFS	Qualitative
	31	Fruit flesh color	FFC	Qualitative-Munsell scale
	32	Fruit number per branch	FIB	Number
	33	Susceptibility to fruit cracking	SFC	Qualitative
Seed	34	Fruit mooring	PFM	Percentage (%)
	35	Seed length	SEL	cm
	36	Seed diameter	SED	cm
	37	Seed texture	SET	Qualitative
	38	Seed ridge ventral view	SVV	Qualitative
	39	Seed form factor	SEF	Value of roundness (0–1)

Fig. 1 *Prunus serotina* Ehrh. probability incidences map in Mexico. Zones in dark have a high statistical probability of exhibiting the presence of the species according to climatic and geographic conditions. Map made with 295 accessions analyzed in FloraMap ver. 1.02



the zones where the three subspecies of *P. serotina* (*capuli*, *serotina* and *virens*) converge; in those areas, adult capulín trees are commonly found in areas without vegetation, probably propagated by means of birds. The presence of adult capulín trees in glades agrees with the reports of Venero (1966) and Avendaño (2000), in which the capulín is considered a pioneer species (Buendía 1988).

Hill & Smith analysis results

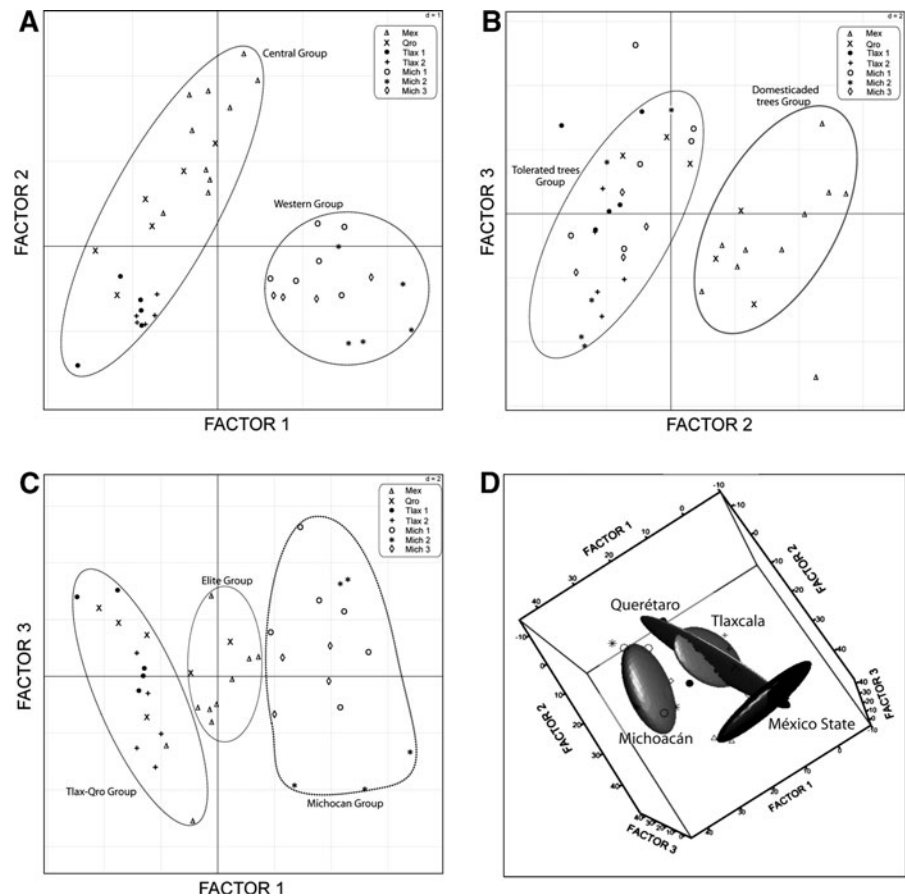
The capulín's morphological variation, described by the Hill & Smith analysis, clearly demonstrates the relationships among the populations studied, as well as the variability present within the study area. The first two principal components (Factors 1 and 2), with the greatest diversity, explained approximately 18% of the total variance in the data set. The first three principal components (Factors 1, 2 and 3) explained 24.5% of the total variance. These results in the multivariate analysis reveal great diversity among the Capulín accessions used in this study.

Two geographical groups of capulín were distinguished (Fig. 2a): the central group, including four populations from three states in the center of Mexico (Tlaxcala, Querétaro and Mexico State), and the western group, including three populations of Michoacán State. This division is a result of geographical factors; morphological differences between the capulín populations from the center and west of Mexico were found, including differences in characteristics

such as productivity percentage (POP), internode length in young stems (ILS), thickness of young stems (TYS), length/thickness quotient length of second basal leaf of flowering branch (LT2), petiole length of second basal leaf of flowering branch (PL2), stamen length (STL), fruit diameter (FRD) and seed diameter (SED). These differences could be a result of the different domestication processes having occurred in the areas, as shown by the differentiation between the cultivated (domesticated) and tolerated trees (Fig. 2b). In the 3D projection (Fig. 2d), three sub-groups were evidenced in the group from central Mexico, with the sub-group *Qro* being an intermediate between the *Mex* and *Tlax* sub-groups. The *Mex* and *Tlax* groups shared morphologically related individuals, mainly according to their fruit and seed size and flower branch length. Most of the individuals with anthropocentrically interesting traits were included in a single morphological group (Fig. 2c). Relationships among the individuals from populations *Mich1* and *Mich2* were evident, but not for the individuals of the *Mich3* population.

We determined correlations among the quantitative variables, such as that between the fruit mooring (PFM) and fruit number per flower branch (FIB) (correlation coefficient: 0.850), the thickness of young stems (TYS) and internode length (ILS) (correlation coefficient: 0.522), and the fruit diameter (FRD) and fruit pedicel length (FPL) (correlation coefficient: 0.736). These results highlight the importance of vegetative growth to the final yield of fruits,

Fig. 2 Visualization of the individuals with respect to three principal components that explain the greater diversity from seven capulín populations' data from Western and Central regions of Mexico. **a** and **b** images show two ecoregions; the groups were formed by States (provinces) of origin in Mexico. **c** This projection shows three groups, separating a group with desirable fruits characteristics (size, color, flavor, and seed size). **d** This projection explains population relationships, showing morphological relationships among Querétaro, Mexico and Tlaxcala populations



which is common in other edible fruit species. The main results of the analysis were the relationships among the qualitative and quantitative variables (Table 3) from the use of the Hill & Smith correlation coefficient (ρ^{HS}). All of the values of ρ^{HS} are positive, and all of the variables have the same direction. For example, the color of the young leaves (YLC) and the tooth number for second leaf border (TQM) both have taxonomic interest, and accordingly McVaugh (1951) used them for his classification and subspecies division of *P. serontina*. The implications of the YLC-TQM relationship and others are addressed in the Discussion section.

Finally, 21 descriptors, such as the determinant for distinguishing capulín populations (Table 4), were recognized based on significant differences for an ANOVA test or Wilcoxon-Kruskal-Wallis test and their importance to botanical distinctness and agronomic characterization. Of these 21 descriptors, 16 were quantitative variables and 5 were qualitative.

However, the list of 39 morphological descriptors can be used for morphological analysis.

Discussion

The present study provides a description of the morphological variability of the capulín in the central and western regions of Mexico and an analysis of the factors affecting their structure in this region. The discussion focuses on the management, use and breeding of the capulín in Mexico.

Distribution of the capulín in the central and western regions of Mexico

We observed that the capulín grows in the ecological niches described by Venero (1966) and Avenaño (2000), that is to say, in a temperate climate, with annual precipitation between 500 and

Table 3 Hill & Smith (ρ^{HS}) correlation coefficients for qualitative variables versus quantitative variables for *Prunus serotina* Ehrh. morphometric data

Qualitative variable	Quantitative variable						
	$(\rho^{\text{HS}} = \alpha \times 10,000)$						
Tree form	ILS	2BL	FRD				
	323	332	90				
Flowering to pruning time	POP	2BT	FBL				
	200	237	481				
Flowering season	TYS	PL2	LPQ	STL	FLD	FPL	FRD
	53	0	4	262	0	359	391
High tree	STL	NFB	FPL				
	123	465	313				
Trunk color	TYS	PL2	STL	FLD	SED		
	40	386	367	104	110		
Leaf upside green color intensity	FIB						
	90						
Nectars color	TQM						
	93						
Young leaves color	POP	2BL	TQM	FPL			
	238	147	467	134			
Fruit form	WITHOUT SIGNIFICANT CORRELATIONS						
Fruit basal form	WITHOUT SIGNIFICANT CORRELATIONS						
Fruit abscission point presence	POP	ILS	TYS	STL	NFB	TFP	FIB
	245	241	97	5	32	276	273
Fruit color	WITHOUT SIGNIFICANT CORRELATIONS						
Thickness of fruit skin	FLD	FRD	TFP				
	360	197	26				
Fruit flesh color	2BT	FPL	FRD				
	100	12	15				
Seed texture	SED	SEF					
	252	330					
Seed ridge ventral look it form	SED	SEF					
	63	0					

1000 mm, with deep soils and in the margins of coniferous forests. In addition, adult trees are commonly found in vegetation clearings, as the capulín is sensitive to shading by taller and more vigorous species (Niembro 1990).

The capulín acts as a pioneering species for perturbed environments, which gives it an additional potential use. The capulíns in central Mexico were close to human settlements, since their fruits and wood are of anthropocentric interest. In the western region, human beings historically did not handle the capulín trees very much, although that appears to be

changing. It is possible to find capulíns in vegetation clearings, gorges, and agricultural fields and on roads. Thus, given the species diversification concepts put forth by Vavilov (1927) and Harlan (1992), the studied regions have the environmental requirements and cultural features to support the diversification of the species.

Avendaño (2000) suggests that capulín diversity is obtained through the continuous processes of diversification and domestication. In a context of improvement and plant breeding *in situ*, as well as conservation, the diversity of the species is

Table 4 Values for the most decisive morphological descriptors; these determined on ANOVA, Wilcoxon-Kruskal-Wallis test and Tukey HSD test and agronomical and botanical criteria

Descriptor	Type	Geographic origin					
		Global		Central group		Western group	
		Mean (μ)	S^2	Mean (μ)	S^2	Mean (μ)	S^2
POP*	Quantitative	43.9189	23.0379	53.8000 A ^a	19.2180	23.3333 B	15.7153
ILS*	Quantitative	1.8641	0.6166	1.5484 A	0.4084	2.5217 B	0.4266
TYS*	Quantitative	0.3538	0.1026	0.3152 A	0.0863	0.4342 B	0.0880
2BL	Quantitative	5.4803	1.0997	5.3240	0.9943	5.8058	1.2764
2BT	Quantitative	2.0338	0.3618	2.0368	0.3275	2.0275	0.4407
LT2	Quantitative	2.7227	0.4756	2.6392	0.4511	2.8967	0.4974
PL2*	Quantitative	1.2432	0.4031	1.1160 A	0.3799	1.5083 B	0.3215
TQM*	Quantitative	8.0270	1.5897	7.5200 A	1.5578	9.0833 B	1.0836
STL*	Quantitative	0.3824	0.0597	0.4140 A	0.0391	0.3167 B	0.0370
FLD*	Quantitative	0.7986	0.1532	0.7416 A	0.1555	0.9175 B	0.0347
NFB*	Quantitative	21.9459	4.5089	23.6400 A	3.3897	18.4167 B	4.6213
FRD*	Quantitative	1.0046	0.3326	1.1084 A	0.3606	0.7883 B	0.0525
TFP*	Quantitative	0.0908	0.0196	0.0968 A	0.0204	0.0783 B	0.0103
PFM	Quantitative	36.7600	15.4981	37.3416	15.0183	35.5483	17.0764
SEL*	Quantitative	0.9684	0.1281	1.0200 A	0.0978	0.8608 B	0.1191
SED*	Quantitative	0.6973	0.0953	0.7456 A	0.0609	0.5967 B	0.0727
HGT*	Qualitative	1.5676	0.9292	1.3200 A	0.9000	2.0833 B	0.7930
FAP*	Qualitative	0.1622	0.3737	0.0400 A	0.2000	0.4167 B	0.5149
TFS*	Qualitative	0.8649	0.5355	1.0000 A	0.5000	0.5833 B	0.5149
SFC*	Qualitative	0.9730	0.6003	1.1200 A	0.6000	0.6667 B	0.4924
SVS*	Qualitative	0.2703	0.5602	0.4000 A	0.6455	0.0000 B	0.0000

Grouped depending on geographic origin (Two groups: Central—*Tlax1*, *Tlax2*, *Mex*, *Qro* and Western—*Mich1*, *Mich2* and *Mich3*)

* Significant differences in ANOVA or Wilcoxon-Kruskal-Wallis test. $p \leq 0.05$. Others selected by botanical or agronomic importance

^a Different letters implicate significant differences between groups with $p \leq 0.05$ for HSD of Tukey test

desirable because (1) diversity allows the application of artificial selection to one or several traits, (2) diversity helps prevent inbreeding, overall, if the species tends to it in commercial production, and (3) diversity supplies material for future breeding programs or in germplasm regeneration programs, e.g. after a catastrophic event.

The diversity reported in this study, along with our knowledge of biological processes such as domestication and other forms of capulín diversification, as well as the traditional knowledge of the species in some regions of Mexico, suggests that the capulín could be considered as a potential commercial crop, supported by a wide genetic base, which could be

useful in modern breeding programs and farmer-participatory plant breeding models.

Morphological variability of Capulín in the central and western region of Mexico

Taxonomic reports identify the *Mich1* and *Mich2* populations as the *serotina* subspecies, whereas the *Mich3* population is reported as the *virens* subspecies (Rzendowski and Calderón de Rzendowski 2005). However, the collation of the morphological information with the McVaugh classification (1951) shows that the correspondence is not exact. Calderón de Rzendowski and Rzendowski (2001) have

reported the possibility of intermediate individuals between the subspecies (intra-specific hybrids). In our analysis, individuals from the Michoacán populations present these characteristics.

Cordeiro et al. (2008) observed a similar morphological pattern in European sweet and sour cherry collections, in which some autochthonous varieties of cherry in Portugal have the morphological characteristics of hybrids between the sweet cherry (*Prunus avium* L. and diploid, AA, $2n = 2x = 16$) and the sour cherry (*P. cerasus* L. and allotetraploid, AAFF, $2n = 4x = 32$). This result makes sense in relation to our observations. Three polyploidy levels for *P. serotina* have been reported (4x, 5x, and 6x) by Dickson et al. (1992) and diploid individuals reported by Forbes (1990). Taking into account that the three species are from the same botanic genus, that the phenomenon of allopolyploidy has been reported for Capulín (Pairon and Jacquemart 2005), and that *P. serotina* is a botanic complex distributed over a wide area, we likely observed intra-specific hybrids.

The phenomenon of hybridization has been considered an important component in the speciation of wild and domesticated plants (Arnold 2004). Recalling Arnold (2004), we are considering natural hybridization to be a phenomenon that implies the occurrence of individuals (hybrids) resulting from a mating between individuals originating in two different populations. Such hybrids are distinguishable from their progenitors with respect to some heritable characters, such as morphological, biochemical, and genetic traits. However, hybrid individuals may be misidentified when the parental groups are morphologically similar to the hybrids and if the distribution of the hybrids overlaps with that of the parental groups (Mahelka et al. 2007).

Hybridization and allopolyploidy may have played important roles in the speciation and colonization of capulín in its native range of distribution and in the environments in which it is considered an invasive species (central Europe). The model of homoploid hybrid speciation can be used in order to understand the morphological intermediacy between groups of related plants (Gross and Rieseberg 2005). However, molecular analyses are necessary in order to verify this application.

We must also consider the influence of human beings on the evolution of capulín. In addition to the ecological speciation and natural selection of capulín, what role has artificial selection played? Is the

“hybrid trait” implied in the domestication process of capulín? Is the “hybrid trait” a target of selection? In order to answer these questions, molecular analysis, and cytogenetic and ethnobotanic techniques implicating the other subspecies of *P. serotina* are desirable. Hybrids are important in plant breeding programs because they are used in the search for new varieties, in understanding the genetic relationships of some traits, and, in the case of *Prunus* species, in agriculture, as rootstocks for fruit tree varieties.

Environmental conditions also influence some characteristics of trees, for example, the action of humidity at the collection sites. The *Mich 2* population is located on the western side of the “Cerro Burro” Mountain (Donkey Hill) in Pátzcuaro, with an agricultural zone bordering the coniferous forest (*Quercus* and *Pinus*). The *Mich 3* population is located in the municipality of Erongarícuaro, in an agricultural zone with stony ground that does not retain humidity efficiently. In addition, the winds circulating around the Pátzcuaro Lake are not favorable. These environmental differences were translated into variable values for the productivity percentage, number of flowers per branch, and length and diameter of the seed, as well as characteristics inherent to the thickness of the fruit’s skin such as POP, number of flowers per branch (NFB), seed length (SEL), SED, thickness of the fruit skin (TFS) and susceptibility to fruit cracking (SFC).

The seed size, from the Tlaxcala populations, suggests that capulíns have been treated and selected by humans on the basis of their seed characteristics at that site, as Avendaño (2000) indicates. In Michoacán, the capulín is used as an edible-fruit species. The *Mex* population presents morphological variation with an agronomic interest. The *Qro* individuals are projected to approximate the *Mex* individuals. Thus, the individuals of Querétaro and Mexico State have a morphological relationship (Fig. 2d). During the collecting trip in Pinal de Amoles, Querétaro, the inhabitants suggested that, 30 years ago, agricultural workers in Puebla State carried capulín seeds with interesting characteristics (flavor, color and fruit size) to Querétaro. This information does not agree with the findings of Rzendowski and Calderón de Rzendowski (2005), who identified the capulín located on this site as members of the *serotina* subspecies.

The morphological variability of capulín in central Mexico likely results from human selection directed

to traits with anthropocentric interest, such as flavor and size of the fruit, as well as the size of the seed. Thus, we can conclude that *P. serotina* is undergoing domestication, as Avendaño (2000) suggests. This interpretation also agrees with that of Cordeiro et al. (2008). They found that more intra-specific morphological variability is found in the sweet cherry (*P. avium* L.) than in the sour cherry (*P. cerasus* L.); they state that this pattern is the product of an intensive domestication process, based on the fruit traits of sweet cherry.

According to McVaugh (1951), samples collected in Querétaro (*Qro*) and Tlaxcala (*Tlax*) cannot be considered as a *serotina* subspecies, because those populations have been selected (*Qro*) and treated (*Tlax*), indicating that their evolution does not correspond to that of organisms undergoing a natural process.

We consider as *treated* plants, those selected plants that are targets of strategies directed to promote their growth and production. These strategies include the elimination of weeds or pruning, for example. Casas and collaborators (2007) provide two terms to refer to this type of *in situ* management of plants under domestication: “Encourage growing” relates to increasing the density of the population (mostly in annual crops), and “Protection,” referring to the deliberate actions favoring the competitiveness of the species under domestication. We consider it convenient to unify these terms, because in a perennial species such as Capulín, before propagation, the people, in the communities that we visited, after selecting the plants, protected them, i.e., they eliminated weeds and branches with symptoms of some diseases or pest infestation. This treatment occurs mostly in the wild or in areas near to agricultural fields with other crops, such as maize, bean or squash.

When the people identified trees showing favorable responses (a greater expression of productivity and/or health), they decided to propagate those plants, by means of seeds or grafting. Commonly, the people decide to move the selected plants and their progenies to an orchard or, occasionally, to their backyards. The management of these plants then changed to an *ex situ* management, in which the expression of characteristics such as fruit yield became the focus of manipulation; we can say that these plants are cultivated plants, because they have

been deliberately produced in order to provide satisfactory products for human consumption.

In the case of the plants from Querétaro, the reason for transferring seeds from a place with selected plants (Puebla) to a place without the species is the desire to get fruits. The implicit reason is propagation of the species, but manipulation for yield is not the main focus yet. Therefore, we can consider that the process of domestication for plants from Querétaro is different from that for populations from Michoacán and Puebla, places in which we could see wild, tolerated, treated and cultivated plants. The plants from Querétaro are evolving from a deliberate transfer, a colonization induced by humans, which involves the introduction of a narrow genetic base. However, these plants are no longer being propagated or manipulated by humans. Additionally, evidence of natural dispersion was seen in Querétaro; it could be a result of the consumption of the fruits by people and birds. Thus, this population can reveal the ecological dynamic of a selected-induced population undergoing colonization under natural selection and the implications for taxonomy.

In contrast, Avendaño (2000) suggests that those populations correspond to *P. serotina* ssp. *capuli*, are undergoing domestication. This suggestion is valid if we consider management as the main criterion for differentiation of the subspecies of *P. serotina*, as McVaugh suggested. However, it is ambiguous in some situations. Therefore, we propose some morphological criteria to differentiate among *P. serotina* subspecies; however, the utilization of additional techniques (e.g. molecular markers in nuclear and organelles and determining of genome sizes) is desirable.

The relationships among the variables

Characteristics such as the thickness of young stems (TYS), internode length (ILS), fruit diameter (FRD), and fruit pedicel length (FPL) mark potential handling strategies, as pruning is of interest for commercial producers. These features indicate to the breeder or agronomist the type of irrigation, tolerance to wind, rain, hail, and birds, some soil types, pruning intensity and support of the fruit. With markers of this type, ways can be devised to exploit the physiology of the species through pruning or some other agronomic techniques of crop physiology manipulation.

Additionally, with such markers, it is possible to assess photosynthesis capability and the ability to be used as a rootstock and determine some of the industrial forestry characteristics, such as wood hardness, wood flexibility, wood porosity, volumetric retractability, compression resistance, traction resistance, and shear stress.

With respect to the quantitative correlations, the variable stamen length (STL) is important because it yields high correlation values for the next variables: the productivity percentage (POP-STL = 0.539), thickness of the fruit pedicel (TFP-STL = 0.534), number of flowers per branch (NFB-STL = 0.505) and seed diameter (SED-STL = 0.534). Descriptors such as the STL, TYS and ILS can be taken as morphological traits for elite individual selection, allowing inferences about the characteristics of yield components and phenotype.

The Hill & Smith correlation coefficient (ρ^{HS}), allows for the introduction of relationships between the quantitative and qualitative variables. For example, McVaugh uses the YLC and TQM at the same time in his taxonomic classification to distinguish subspecies. Our analysis demonstrates that both variables give the same information; however, these are different types of variables (YLC = qualitative, TQM = quantitative). Relationships of this type make it possible for the researcher to choose convenient variables because they avoid the duplication of information. Relationships like the one between flowering season and fruit diameter (FWS – FRD) show that, though they give similar information, it is important to take into account traditional knowledge. The settlers of the zones adjacent to the capulín populations indicated the variable FWS, whereas the authors determined FRD. Situations of this type justify the use of multivariate methods with variables of different natures and origins. These methods permit us to maximize the usefulness of the information, linking the scientific and the traditional knowledge. This model can be applied to other underutilized species for which traditional knowledge is available.

The variables showing important differences among the states, localities and populations are the petiole length of the second basal leaf of the flowering branch (PL2) and the number of teeth in the margin of the second basal leaf of the flowering branch (TQM). It is these two variables that McVaugh (1951) used to describe the subspecies.

The stamen length, flower diameter, number of flowers per branch and thickness of fruit petiole, seed length and equatorial diameter of seed (STL, FLD, NFB and TFP, SEL, SED, respectively) variables are good descriptors of the differences between the groups. The first four variables are always used in taxonomic classifications. Variables such as SEL and SED are important for germplasm characterization because seed variables are usually stable in time and space (González-Andrés and Ortiz 1995).

The list of descriptors for cultivated species includes quantitative and qualitative characteristics. Those of Cordeiro and his collaborators (2008), who have used both types of characteristics in their analyses, are important for an understanding of the role of native and autochthonous germplasm in intra-specific variability. Methods that permit us to analyze both types of variables at the same time, and the relationships among the variables, are important in providing adequate scientific support for the results, easy interpretations of them, and maximization of the information from plant material with anthropocentric relationships.

Implications for the genetic resource management of the capulín

The capulín is a fruit-bearing tree widely distributed throughout the central-western region of Mexico. From our results, two ecoregions are differentiated: the populations from Tlaxcala, Querétaro and México State comprise the central region, where the capulín is used to produce fresh fruits and seeds for consumption, and the western region (Michoacán), where the species provides seeds for consumption, fresh fruit and agro-ecological services.

We consider agro-ecological service to be the direct or indirect benefits resulting from incorporating a native species under domestication into the agro-ecosystem. In the case of the capulín, maize producers from the area of Pátzcuaro use it as a windbreak, but they have also selected trees fructifying in August (out of season) for their windbreak walls. The consequence of this deliberate action is that the birds prefer to consume capulín fruits rather than the maize grain (a base for human food), because the fruits are bigger, sweeter and more easily available than the maize grains. The implication of this use of the capulín is that the losses in maize yield are less than

would occur when the capulín is not present in the vicinity of the maize fields; therefore, the capulín acts as a distracter for potential pests. Thus, the phenotypes of capulín exhibiting delayed fructifying are valuable, and their management and conservation is part of the traditional knowledge of the community today.

The capulín seeds are considered to be orthodox with latency (Muratalla 1984; Camacho 1980). Their loss of viability has not been evaluated. The clonal propagation of capulín is easy through grafting onto another capulín tree. *In situ* conservation of capulín in Mexico is necessary. Our work contributes to the descriptions of individuals having special morphological characteristics for agronomic purposes and to the methods for analyzing these characteristics. *In situ* conservation is urgently needed and recommended for the Michoacán populations. The wild variation is valuable, but it is threatened by the increasing introduction of germplasm from the central region, because such fruit characteristics as fruit size, flavor and color are different in the two populations. Moreover, agricultural land division is severely disturbing the environment of the species being developed.

The genetic relationships among the subspecies of capulín have not yet been clarified. Forbes (1990) suggests the possibility of weak gametophytic self-incompatibility. Dickson et al. (1992) report polyploidy at different levels for capulín. The morphological, physiological and biochemical variations of some subspecies seem to be related to polyploidy. The allopolyploidization process could explain the presence of individuals with characteristics of different subspecies. Additionally, epigenetic phenomena such as mutations are recurrent in the allopolyploidization and could be considered part of the evolutionary process in capulín. Pairon and Jacquemart (2005) reports values of observed heterozygosity (H_o) of 0.987 and an average number of alleles per locus (A) of 10.33 for *P. serotina* (probably ssp. *serotina*). These suggest that the capulín is a highly heterozygotic species with allopolyploid origins, explaining its tetraploidy.

The populations from central Mexico exhibit important morphological similarities, possibly as a consequence of human selection. The evolution of populations from the western Mexico region has been less linked to anthropocentric interest related to fruit

characteristics, in comparison to populations from central Mexico; instead western Mexico populations appear to be undergoing an incipient domestication process.

The 39 morphological descriptors used could be useful in the agronomic description of the capulín. Descriptors such as the stamen length (STL), thickness of young stems (TYS) and internode length (ILS) have been linked with fruit quality variables. These descriptors can be used as indirect morphological traits for the selection of individuals for breeding programs.

The morphologic variability of the capulín in the central-western region of Mexico described in this study suggests a wide genetic base, useful for improving this species by genetic breeding. Breeding could be directed to the obtaining of selections with better fruit or seed quality, rootstocks for other related species and forestry uses.

Ex situ conservation for the capulín is possible because of the characteristics of its seeds, as well as its simple propagation through grafting. Some communities in Michoacán and Puebla have adopted the practice of grafting as a method for propagating plants with big, sweet fruits, but it has not been generalized to the establishment of orchards. Methods such as cryoconservation and the development of synthetic seeds and cell suspensions must be evaluated. *In situ* conservation is urgent for the western populations, due to the changes in the environment of the species during its development and the introduction of germplasm from the central region of Mexico. These changes imply the loss and displacement of wild germplasm and tolerated individuals. For both types of conservation, our results can be used to help in the selection of individuals and sites.

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