Article

Morphological characterization of *Agave angustifolia* and its conservation in Guerrero, Mexico

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Abstract

Agave angustifolia has a wide morphological variation, which often makes its taxonomy difficult. The differentiation between *Agave* species is made from homologous and independent morphological characteristics, whose variation can be ontogenetic and genetic. The objective of this research was to characterize the morphology of *A. angustifolia* called ‘sacatoro’ from the state of Guerrero. The research was conducted from 2016 to 2017, in six populations of *A. angustifolia* and two populations as control, *A. angustifolia* Haw and *A. cupreata*. 32 stem and leaf variables of mother plants and shoots were measured for each specimen, according to the technical guide for the varietal description of *Agave* spp. The data were analyzed using main components (ACP) and hierarchical agglomerative grouping (AJA). The variables that allowed to discern the populations were: shape, edge, color and texture of the leaf, striae on lateral spines and length of the terminal spine, all with a positive correlation. The results obtained from the ACP and the AJA allowed observing the relationship between variables and specimens based on the morphological attributes shared by the species studied. Despite the morphological variation between maguey sacatoro and *A. angustifolia* Haw, a close positive relationship between both is shown. So, it is inferred that the phenotypic characteristics that subgrouping the maguey sacatoro, could show as a result a possible variant of *A. angustifolia* Haw that would allow to locate taxonomically this group to contribute to the ordering of the *Agave* genus and its conservation.

Keywords: *Agave angustifolia*, conservation, maguey sacatoro, phenotypic.

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Introduction

The agave, commonly called 'maguey', is a plant whose leaves, stems, flowers and fruits are used to satisfy various needs as a source of food, drink, medicine, ornaments, fiber, fuel and fertilizer. In addition, the floral scape and its fibers are used for the construction of houses, as an agricultural implement and flowers are highly valued in human nutrition (García-Mendoza, 2007). In general, it offers more than 100 forms of use, among which 70 serve as employment to the people who give it some kind of use. Of these, the production of distilled beverages such as: bacanora, charanda, sotol, tequila and mezcal stands out (Esparza-Ibarra *et al*., 2015).

The magueyes are plants that have social, economic and ecological importance, that thrive in diverse ecosystems and altitudes. Its center of origin is Mexico, although it is also distributed to the southwest of the United States, Central America, the Caribbean and northern South America (Vázquez-García *et al*., 2007). 211 species are recognized, of which 159 (75%) are located in Mexico and of these 119 (57%) are endemic (Garcia-Mendoza, 2012). Therefore, there is a high diversity in the Agavaceae family (García-Mendoza, 2007; Jacinto, 2013).

The high number of endemic species in Mexico suggests that the diversity of the group is due to the physiology that has allowed local adaptation in heterogeneous habitats and this process of diversification has probably been accepted by the domestication to which several species have been subject of Agavaceae (García-Mendoza, 2007). The wide ecological tolerance, the dispersion and germination of seeds and the interaction with other organisms (pollinators), depends on the intrinsic properties of the genus such as genetic diversity (García-Mendoza, 2002).

The plants of the genus *Agave* are monocotyledonous, their leaves are shown in a rosette arrangement, also known as pencas, depending on the species, the edge of the pencas can be linear, spatulate, lanceolate, deltoid, oblong or ovate, they are generally rigid, in their most with lateral spines (Gentry, 1982; García-Mendoza, 2007). Its stem, depending on the species can be acaulescent or caulescent, is also called 'heart of the maguey or pineapple of maguey'. It is a hermaphrodite plant, which has an inflorescence in spike. Its flowers are greenish yellow. Its fruit is woody capsule elongated dehiscent and each capsule contains numerous flattened seeds and black testa (Esparza-Ibarra *et al*., 2015).

A. angustifolia Haw., Also known as 'thin maguey' or 'espadín', is the source of fiber and alcoholic beverages such as bacanora and mezcal (Vazquez-García *et al*., 2007). This maguey is distributed mainly in Mexico, in the states of Aguascalientes, Campeche, Chiapas, Chihuahua, Durango, Guerrero, Jalisco, State of Mexico, Michoacán, Morelos, Nayarit, Oaxaca, Puebla, Quintana Roo, Tamaulipas, Veracruz, Yucatan and Zacatecas from sea level to more than 2 000 masl (Vázquez-García *et al*., 2007).

Also, García-Mendoza (2011) mentions that its distribution is located to Panama. Therefore, it is the species with greater distribution within the *Agave* genus, due to its morphological diversity and adaptability. This species is part of the *Agave angustifolia* complex, which has allowed its taxonomic reclassification in more than 20 different species (CONABIO, 2006; García-Mendoza and Franco-Martínez, 2017).

For the state of Guerrero there is no research on maguey sacatoro, so the need arises to characterize it morphologically to have information that allows to know with greater certainty if it is possible to differentiate it from other populations of *A. angustifolia* Haw. This being the purpose of the study, since this population of maguey is important because it is used as a raw material in the production of mezcal in the state of Guerrero, since about 1.7 million liters of mezcal are produced annually, which represents an important source of income for Guerrero families, related to this activity (Kirchmayr *et al*., 2014).

The mezcal product of the maguey sacatoro has denomination of origin; its production process specifically in the cooking of maguey, grinding, fermentation and distillation groups it in the category 'artisanal mezcal' which has allowed it to have very particular organoleptic characteristics and therefore its demand at the international level has increased. Knowing the morphological diversity will be useful to control the quality and at the same time conserve the germplasm and in general contribute in the ordering of the *Agave* genus, for the economic importance that this species has in the elaboration of mezcal.

Materials and methods

Study area

The research was conducted in the northern and central regions of the state of Guerrero; specifically, in four municipalities: 1) Huitzuco de los Figueroa; 2) Atenango del Río; 3) Chilapa de Álvarez; and 4) Ahuacuotzingo; in which eight locations were studied: 1) Atetetla; 2) Paso Morelos; 3) Coacan; 4) Los Amates; 5) Ayahualco; 6) Santa Cruz; and 7) Motuapa and old Trapiche (Figure 1).

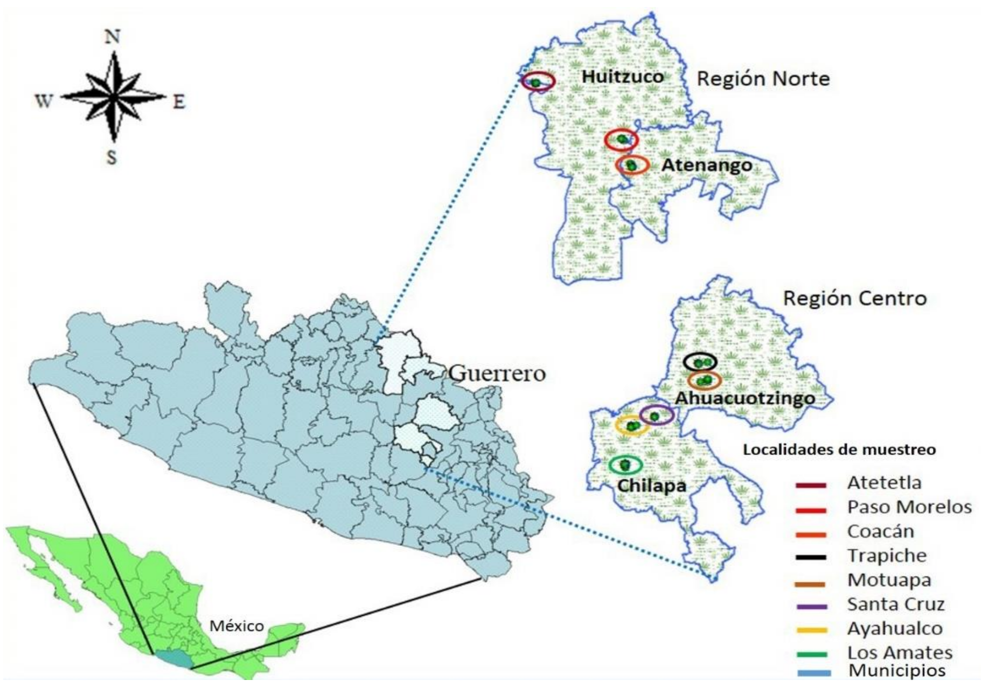

Figure 1. Geographical location of the study area, municipalities of Huitzuco de los Figueroa, Atenango del Río, Ahuacuotzingo and Chilapa de Álvarez, Guerrero, Mexico.

The selection of localities was made based on the existence of maguey populations, in their wild form (Los Amates locality) or cultivated (all others), are localities that produce mezcal. They were georeferenced with respect to their latitude, longitude and altitude of each sampling site, it was carried out with a global positioning system (GPS), Garmin brand, model eTrex 30. The altitude ranged between 800 and 1 500 m (Table 1).

Table 1. Geographic location of the *Agave* sampling locations in Guerrero.

Agave	Location	Species key	Municipality	Latitude (N)	Longitude (W)	Altitude (m)
A. angustifolia (maguey sacatoro)	Atetetla	HAT	Huitzuco	18°19’43.4’’	99°23’57.7’’	892
A. angustifolia (maguey sacatoro)	Paso Morelos	HPM	Huitzuco	18°12’50.5’’	99°12’26.6’’	1 107
A. angustifolia (maguey sacatoro)	Coacan	ARC	Atenango	18°09’44.2’’	99°11’24.2’’	1 029
A. angustifolia (maguey sacatoro)	Los Amates	CLA	Chilapa	17°32’25.3’’	99°11’54.6’’	1 476
A. angustifolia (maguey sacatoro)	Motuapa	AMO	Ahuacuotzingo	17°44’57.5’’	99°02’15.1’’	1 308
A. angustifolia (maguey sacatoro)	Trapiche	ATV	Ahuacuotzingo	17°42’42’’	99°00’56.7’’	1 295
A. angustifolia Haw (maguey espadín)	Sta. Cruz	CSC	Chilapa	17°37’15.7’’	99°11’11’’	1 474
A. cupreata Trell & Berger (maguey papalote)	Ayahualco	CAY	Chilapa	17°37’2.6’’	99°11’7.4’’	1 534

Phenotypic descriptions of the maguey plant were carried out (n= 57). In the sampling sites, the technical guide was used for the varietal description of *Agave* spp. (SNICS, 2014). 32 variables were evaluated for each specimen, the description of the variables measured in: plant, stem, leaf and suckers of each maguey are:

- Habit of growth of the plant: 1) acaulescent, 2) caulescent
- Height of the plant: 3) low, 5) medium, 7) high
- Diameter of the rosette: 3) small, 5) medium, 7) large
- Number of leaves of the plant: 3) low, 5) medium, 7) high
- Number of sheets per filotaxis: 3) low, 5) medium, 7) high
- Stem visibility: 1) absent, 2) present
- Length of the sheet: 3) short, 5) medium, 7) long
- Width of the blade: 3) narrow, 5) medium, 7) wide
- Relationship between length and width of the leaf: 3) small, 5) medium, 7) large
- Leaf shape: 1) linear, 2) spatulate, 3) deltoid, 4) lanceolate, 5) oblong, 6) ovate
- Form of the cross section of the leaf: 1) flat, 2) V-shaped, 3) U-shaped, 4) concave, 5) keel, 6) obdelated, 7) oblate, 8) hemioblate, 9) circular
- Curvature of the leaf: 1) absent, 2) recurved, 3) curved, 4) corrugated

- Edge of the leaf: 1) smooth, 2) corrugated, 3) serrated, 4) crenate
- Texture of the leaf: 1) smooth, 2) rough
- Glaze of the leaf: 1) absent, 2) present
- Leaf color: 1) green, 2) green yellow, 3) blue
- Intensity of leaf color: 1) weak, 5) medium, 7) strong
- Secondary leaf color: 1) absent, 9) present
- Tone of the second color of the leaf: 1) yellow, 2) white
- Distribution of the second color: 1) marginal, 2) central, 3) reticulated
- Lateral spines of the leaf: 1) present, 9) absent
- Shape of the lateral spines: 1) straight, 2) curve, 3) hooked, 4) filifera
- Profile of lateral spine: 1) monofurcated, 2) bifurcated, 3) trifurcated, 4) polyfutured
- Color of the lateral spines: 1) white, 2) brown, 3) reddish, 4) black
- Uniformity in the size of the thorns: 1) homogeneous, 9) heterogeneous
- Number of lateral spines of the leaf: 3) few, 5) means, 7) many
- Distance between the lateral spines of the leaf: 3) short, 5) medium, 7) long
- Stretch marks on the lateral spines of the leaf: 1) absent, 9) present
- Form of the terminal spine of the leaf: 1) straight, 2) curved, 3) filiform, 4) poly-shaped
- Length of the terminal spine: 3) short, 5) medium, 7) long
- Prolificity of children: 1) absent, 2) low, 3) medium, 4) high
- Cycle to started flowering of the plant: 3) early, 5) intermediate, 7) late

From the 57 determined specimens, 1 824 data were obtained, with which a homogenization of the 32 variables evaluated was carried out based on the technical guide (SNICS, 2014). The data were subjected to Pearson correlation analysis with significance level of $p= 0.05$, as a measure to identify the relationship or association between randomly selected variables. According to Castañon-Najera *et al*. (2008), the variables that contribute little or nothing to the explanation of the two-dimensional plane were eliminated.

Therefore, the variables habit of plant growth was eliminated; number of leaves per filotaxy, visibility of the stem, width, curvature, secondary color, shade of the second color and distribution of the second color of the leaf, lateral spines, profile of lateral spines, prolificacy of shoots and cycle at the beginning of flowering. Subsequently, the principal components analysis (PCA) was carried out. The ACP aims at the transformation within a system of relevant coordinates and the reduction of the dimension. With this analysis the correlated variables are taken, which describe observations and a linear combination is found to generate new uncorrelated variables, called main components (CP or PC), so the ACP was generated with the 20 variables that provided the most explanation.

That is, 12 variables with very low significance and high correlation values were discarded. This reclassification of variables was made based on the correlation, with the purpose that the variables involved in the analysis had the same importance (Crossa *et al*., 1995). The significance of the eigenvalues was determined by the Keizer rule (1960). An agglomerative hierarchical clustering analysis (AJA) was also performed, using the UPGMA linkage method, the measure of dissimilarity was the Euclidean distance. Statistical analyzes were performed with the XLSTAT Software (Fahmy, 1993).

Results and discussion

The species *A. angustifolia* grows in various types of vegetation, from coastal dunes to oak forests, with phenotypic variation depending on the environment it occupies, resulting in the intra-specific taxonomy of *A. angustifolia* being complicated (García-Mendoza and Chiang, 2003). However, although the maguey sacatoro belongs to the *A. angustifolia* complex, the population has phenotypic characteristics that differentiate it from *A. angustifolia* Haw. To characterize *A. angustifolia* morphologically, a total of 57 agave specimens were evaluated, of which 47 were of *A. angustifolia* sacatoro, 10 of *A. angustifolia* espadín and 10 of *A. cupreata* papalote, of which important variations were observed between specimens and between populations (Table 2).

Principal component analysis (PCA)

In Table 2, it is shown that the first six CPs explain 79% of the total variation in the data. Therefore, they generate basic knowledge about the relationships between variables and main components. The CP1, explains 30% of the total variation with eigenvalue of 5.976, while the CP2 22% of the variation with eigenvalue of 4.385. CP3, CP4, CP5 and CP6 explained 9.647, 6.774, 5.874 and 4.874% of the morphological variation with eigenvalues of 1.929, 1.355, 1.175, and 0.975, respectively.

Table 2. Eigenvalues between morphological attributes of agaves: the first six CP.

Variables	CP1	CP2	CP3	CP4	CP5	CP6
Height of the plant	-0.135	-0.103	0.471	-0.156	0.007	0.001
Diameter of the rosette	-0.079	-0.08	0.485	-0.358	0.061	0.072
Number of leaf	-0.091	0.278	0.238	-0.198	-0.243	-0.091
Length of the leaf	-0.169	-0.145	0.445	0.171	-0.305	-0.043
Relation length and width of the leaf	-0.13	0.085	0.236	0.694	-0.001	-0.054
Form of the leaf	0.324	-0.113	0.007	-0.06	-0.222	0.157
Form of the cross section of the leaf	0.079	-0.201	-0.015	0.032	0.396	0.269
Edge of the leaf	0.394	-0.055	0.119	0.05	-0.001	-0.009
Leaf texture	0.225	-0.376	-0.052	-0.065	-0.071	-0.104
Glaze of the leaf	0.111	0.417	0.185	0.132	0.088	0.122
Leaf color	0.381	-0.044	0.077	0.045	0.03	-0.014
Intensity of leaf color	-0.036	-0.186	0.125	0.029	0.123	0.786
Lateral spine shape	0	0.25	0.081	-0.309	0.56	-0.187
Color of lateral spines	0.193	0.277	0.021	-0.315	-0.195	0.131
Uniformity in the size of thorns	0.111	0.417	0.185	0.132	0.088	0.122
Number of lateral spines	-0.183	-0.311	0.133	-0.168	-0.102	-0.16
Distance between lateral spines	0.297	-0.051	0.182	0.027	0.024	-0.042
Stretch marks on lateral spines	0.361	-0.008	0.126	0.046	-0.034	-0.272
Terminal spine shape	-0.023	0.232	-0.195	-0.141	-0.483	0.268
Terminal spine length	0.379	-0.051	0.115	0.052	-0.065	0.019
Autovalue	5.976	4.385	1.929	1.355	1.175	0.975
Variability (%)	29.882	21.925	9.647	6.774	5.874	4.874
Cumulative variability (%)	29.882	51.807	61.454	68.228	74.101	78.975

In the two-dimensional plane (Figure 2), group I is made up of specimens of *A. angustifolia* 'sacatoro' and group II, by specimens of *A. angustifolia* Haw 'espadín' with positive correlation between them. That is, groups I and II have a strong correlation, so it tends to be located and grouped towards the left end of CP2 in the two-dimensional plane and group III formed by specimens of *A. cupreata* Trell & Berger 'papalote', sample a positive correlation between specimens, but negative with respect to groups I and II.

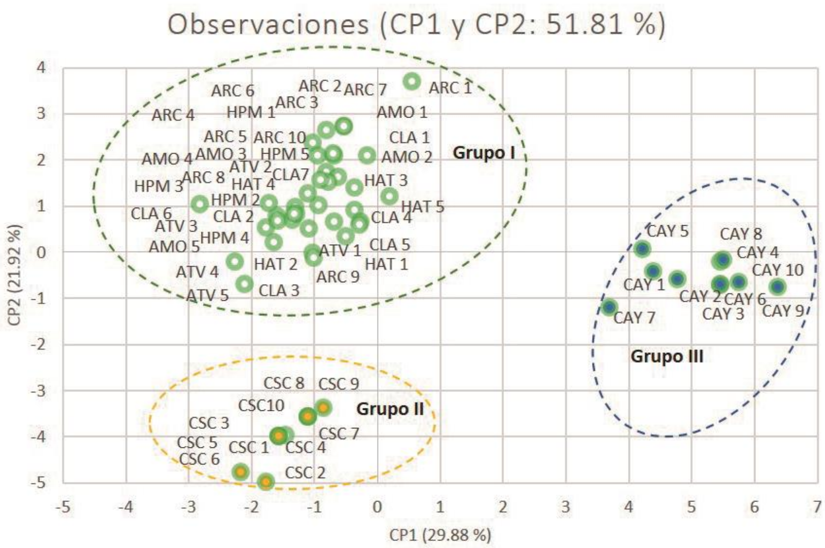

Figure 2. Two-dimensional plane of CP1 and CP2. Group I (maguey sacatoro). Atetetla (HAT); Paso Morelos (HPM); Coacan (ARC); The Amates (CLA); Motuapa (AMO) and Trapiche Viejo (ATV). Group II (maguey espadín). Santa Cruz (SCS). Group III (maguey papalote). Ayahualco (CAY).

The CP1, was determined in a positive and significant way by the shape, edge and color of the leaf, striae on the lateral spines, distance between lateral spines and length of the terminal spine and negatively influenced the number of lateral spines, length of the leaf and the height of the plant. The CP2, is determined positively and significantly by the glaze of the leaf, uniformity in the size of the thorns, color of the lateral spines, number of leaves and shape of lateral spines, in the same component influenced negatively the texture of the leaf and the number of lateral spines.

The CP3, is formed by diameter of the rosette, height of the plant and length of the leaf, while the CP4, CP5 and CP6 are formed by a single variable, relationship between length and width of the leaf, shape of lateral thorns and intensity of the leaf color, respectively. It is worth mentioning that only the graphical results of CP 1 and 2 are presented, because it is visually complicated to understand the analysis with the 6 CPs.

Mora-López *et al*. (2011), took into account quantitative variables such as height and diameter of the rosette, length of the leaf, number, length, and distance between the lateral spines, which allowed them to separate populations of the genus Agave. Rivera-Lugo *et al*. (2018) in its research on the morphological and genetic variation of the *A. angustifolia* complex, showed useful traits for the taxonomic identification of this species and shows, with a phenotypic dispersion, that the taxa are clearly grouped according to the level of cultivation and management that is it gives to the plant. However, qualitative variables, although they are little influenced by environmental conditions, are also determinant (Franco and Hidalgo, 2003).

Although *A. angustifolia* 'sacatoro' and *A. angustifolia* 'espadín' show a positive correlation with each other, it is clear that maguey espadín is distinguished by its greater domestication. This distinction of this species is due to the fact that it shows larger leaves and higher plant height, in contrast to the results, taxonomic implications of the morphological and genetic variation of the populations of the *A. angustifolia* de Rivera-Lugo complex (2018), in this work was not considered as a reproductive stage, but in the field trips it was possible to corroborate that the inflorescence and terminal spine compared to the maguey sacatoro is larger. Rodríguez-Garay *et al*. (2009), determined with an ACP study the relationship between *A. tequilana* var. Azul and *A. angustifolia* var. Lineño, obtaining a total variability 79% similar to that obtained in this study using the same technique, but with *A. angustifolia* 'sacatoro' and 'espadín'.

Figueredo *et al*. (2014) through multivariable methods, based on morphological characters, differentiated populations of wild and cultivated maguey reflecting artificial selection; showing that *A. hookeri* could be the end of a domestication gradient in a species complex, due to the similarity it presents with cultivated *A. inaequidens*. On the contrary, wild magueys such as *A. macroculmis* are small plants with large spines. Taking into account that the mechanical protection of the lateral spines is an important characteristic in the plant towards its predators, due to the incipient management conditions in which it is found (Castañon-Najera *et al*., 2008).

In the same way, the maguey sacatoro, collected in the locality Los Amates in its wild form, has a small rosette (80 cm high) and small and numerous lateral spines (more than 150 lateral thorns) in comparison with the specimens evaluated in the Atetetla, Paso Morelos and Motuapa localities with an incipient management, which have a plant height of more than two meters, large lateral spines and in smaller quantity (from 60 to 80 lateral thorns in the leaf).

That is why, when a population of specimens is characterized in a morphological way and we work with quantitative and qualitative variables, it is important to reclassify variables based on correlation or multivariate normality tests, applying the central limit theorem, it is understood that samples comprise a 'normal' population (Castañon-Nájera *et al*., 2008). The distribution and grouping of the specimens were complex; therefore, it was decided to consider a multidimensional analysis to form classes or groups defined by similarities as well as by morphological dissimilarities (Figueredo *et al*., 2014; Avendaño-Arrazate *et al*., 2015).

Analysis of agglomerative hierarchical clustering (AJA)

The agglomerative hierarchical clustering analysis (AJA) represents the way in which the algorithm groups individuals from the Euclidean distance and then the subgroups, from the cofenetic distance. Finally, the algorithm regroups the observations progressively. The dotted line presents the truncation and shows the grouping defined by the Euclidean and cofetic distances in three groups, represented by maguey sacatoro (group I), maguey espadín (group II) and maguey papalote (group III).

Group III is shaped by a maguey papalote in a rearrangement of the subgroups with a cofeetic distance of 317. Group II is formed by maguey espadín, at a distance of 219 and the group I, consisting of maguey sacatoro. Although group I is well differentiated from group II and III at a dissimilarity distance of approximately 75%, it is divided into at least two subgroups; subgroup A, at a cofeetic distance of 35, consisting of 16 specimens: ARC1, HAT3, HAT5, AMO2, CLA4, CLA5, ARC6, AMO1, ARC2, ARC7, HPM1, ARC5, HPM5, ARC10, ARC4 and CLA1 and the subgroup B, at a cofeetic distance of 49 made up of 21 specimens: HPM3, HPM2, CLA7, HAT2, ATV3, ARC8, HPM4, ATV4, ATV5, CLA6, ARC9, CLA2, AMO4, AMO5, HAT1, CLA3, ATV1, ARC3, AMO3, HAT4 and ATV2, which confirms the grouping of the ACP (Figure 3).

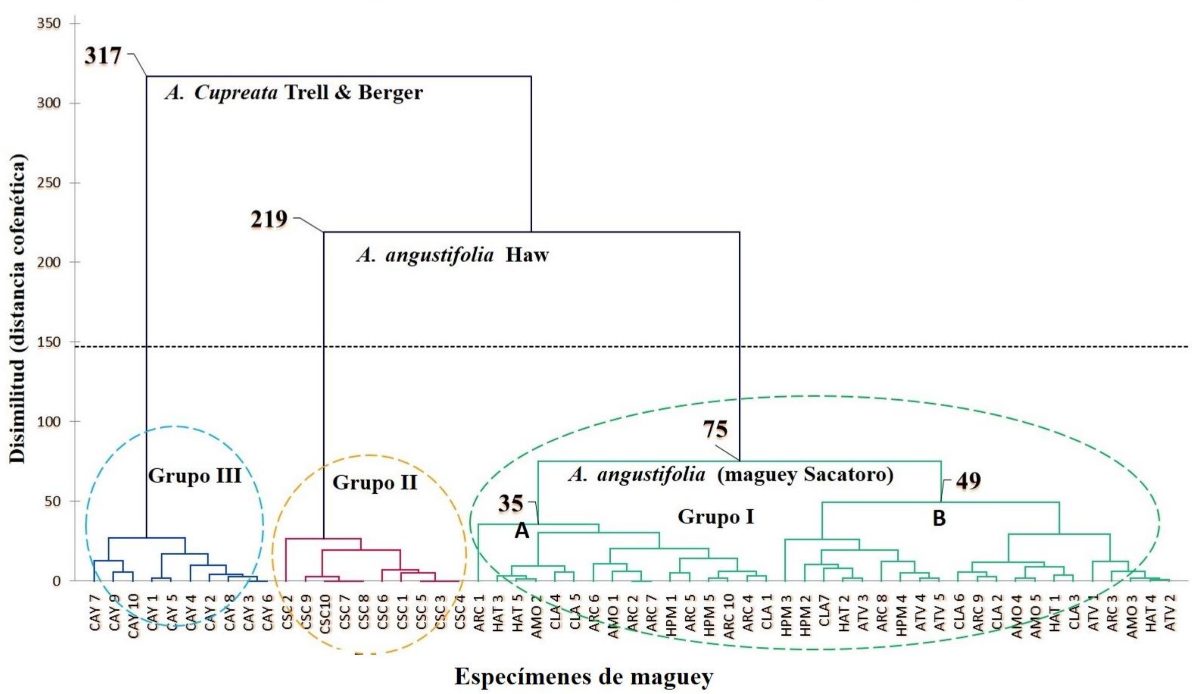

Figure 3. Dendrogram of maguey specimens in three groups: *A. angustifolia* Haw (maguey espadín), *A. angustifolia* (maguey sacatoro) and *A. cupreata* (maguey papalote).

The specimens of *A. angustifolia* sacatoro, although they are located in a separate subgroup, are also part of group II that makes up *A. angustifolia* 'espadín'. Said sub-grouping is due to the type of management of the plant. The agaves evaluated in the field and some of their characteristics with which the ACP analysis and the AJA analysis were performed are observed in the (Figure 4 and 5).

The analysis of the main components and hierarchical grouping were useful tools for grouping the specimens according to the similarities or dissimilarities of the morphological variables of the agaves studied. In addition, in other investigations, using quantitative and qualitative characteristics through ACP analysis, the morphological and genetic relationships of this species have been identified (Colunga-García *et al*., 1996; Castro-Castro, 2010). However, according to Rodríguez-Garay *et al*. (2009), a genetic analysis, would yield more information about the morphological variation among the populations evaluated.

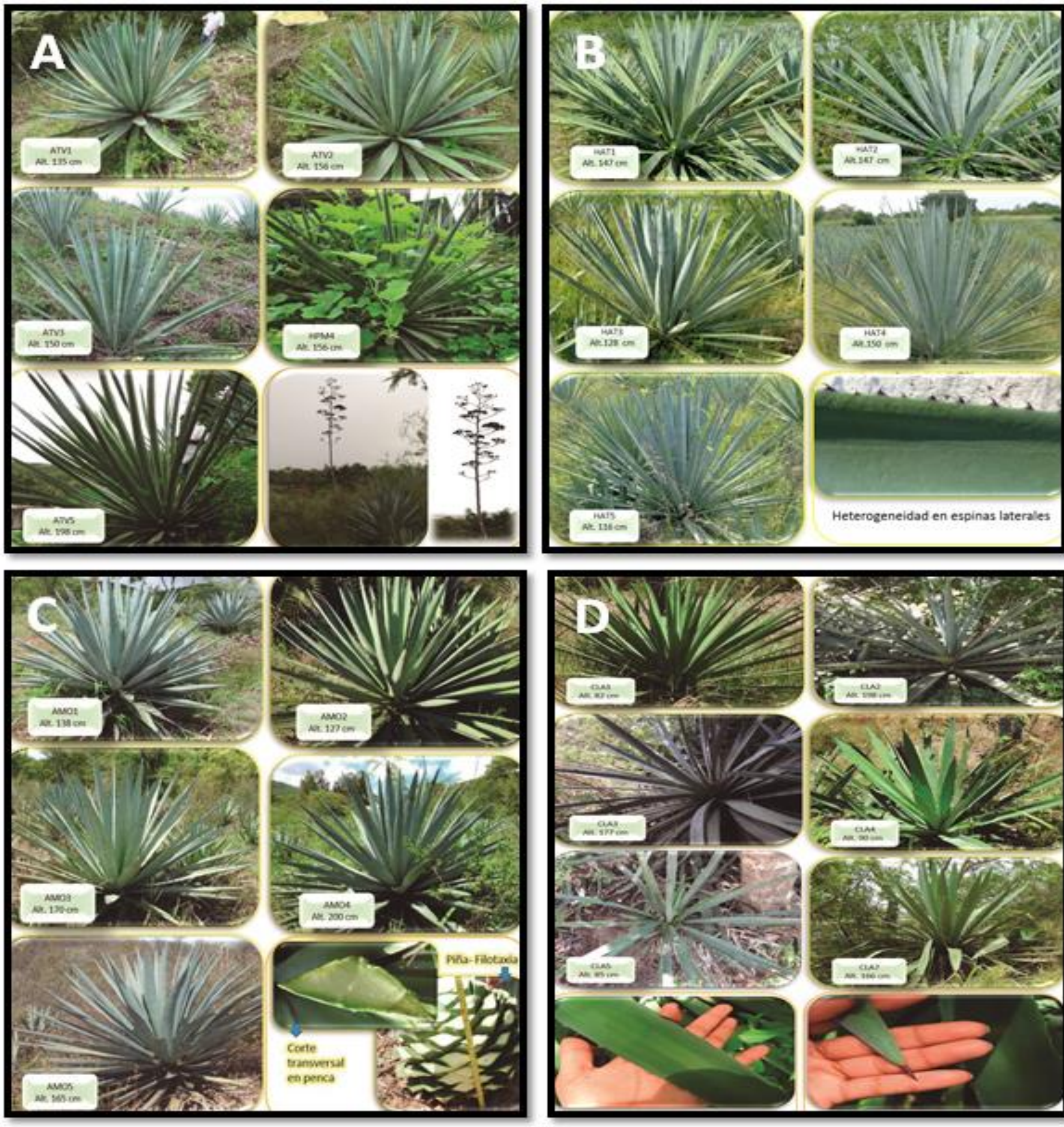

Figure 4. Taxon *A. angustifolia* 'psacatoro' and sampled localities: A) Trapiche Viejo; B) Atetetla; C) Motuapa; and D) The Amates.

The results of the evaluated variables served to determine the morphological characteristics of the taxon *A. angustifolia* 'sacatoro'. According to Narez-Jiménez *et al*. (2014), the demand for raw material to be able to maintain the production of mezcal in the cultivated plants indicates that the magueyes constitute a valuable genetic resource that deserves to be studied to improve its use and conservation.

Figure 5. Taxa and localities sampled. A) *A. angustifolia* 'sacatoro', locality Paso Morelos. B) *A. angustifolia* 'papalote', locality Ayahualco; C) *A. angustifolia* 'sacatoro', locality Coacan; and D) *A. angustifolia* 'espadín', locality Santa Cruz.

Conclusions

The morphological analyzes carried out showed differences between variables and taxa studied. The characters of intensity of color of the leaf, shape of lateral thorns, relation between length and width of the leaf, diameter of the rosette, height of the plant, length of the leaf, uniformity in the size of the thorns, length of the terminal spine, leaf color, leaf shape, distance between lateral spines, number of leaves and number of lateral spines, together are the most important in the morphological delimitation of taxa.

The taxa *Agave angustifolia* 'sacatoro' and *Agave angustifolia* 'espadín', are finally the same species. Although both can be distinguished morphologically, the results confirm a close relationship between them. Therefore, the morphological differences could be attributed to the vegetative selection and propagation of the germplasm with desirable characteristics due to the excessive use as raw material for the production of mezcal, main use in the study area.

Acknowledgments

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