

Morphometric characterization of mezcal agaves from the Northern Region of Guerrero

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Abstract

The current knowledge of farmers in the Northern Region of Guerrero about the identification of agave species and varieties used in mezcal production is limited. By not knowing the scientific name of these species, they are prevented from accessing information on forms of reproduction, management and byproducts, as well as the certification processes of agave plantations for mezcal production. This research aimed to characterize the morphometry of mezcal agaves and identify traits that can be used for their identification. The research was conducted from 2016 to 2023, collecting 230 agave specimens in 31 localities in the Northern Region of Guerrero. Twenty-three morphometric traits were measured, which were entered into a database in Microsoft Excel[®]. Principal component analysis and linear discriminant analysis were performed in the R software version 4.0.3. The first five principal components explained 79.59% of the variation. Linear discriminant analysis required two discriminant functions to explain 89% of the variation. The traits that contributed the most in the principal component analysis were tooth uniformity, leaf margin, and length of tooth in the middle of the leaf. In linear discriminant analysis, the traits that contributed the most were wider leaf width, leaf length, and leaf base width. The identification of these species and varieties will make it easier for farmers to certify their agave plots and give added value to the mezcal.

Palabras clave:

agave, linear discriminant analysis, morphometrics, principal component analysis.

Introduction

The genus *Agave* belongs to the family *Asparagaceae*, which is endemic to the Americas (Chávez-Parga *et al.*, 2016). The countries with the highest record of *Agave* taxa are Mexico, the United States of America, Cuba, and Guatemala. Mexico is the center of greatest richness and diversity of these plants, where 159 species are recorded, of which 129 are endemic (García-Mendoza *et al.*, 2019). There are records of the domestication of agaves by Mesoamerican settlers dating back approximately ten thousand years, who used them as food, housing construction, clothing, fiber, beverages (such as tequila or mezcal) and medicine (Álvarez-Duarte *et al.*, 2018).

At present, agaves are still of great cultural and economic importance to Mexicans as almost all their morphological structures are used, both from wild and domesticated species (Delgado-Lemus *et al.*, 2014). Several authors suggest the need to carry out morphometric, molecular, and genetic studies in order to obtain a more appropriate classification and identification for the delimitation of these taxa (Barrientos-Stream *et al.*, 2019; Fragoso-Gadea *et al.*, 2021).

However, a problem that arises when identifying agave species used in the production of mezcal is the definition of diagnostic traits that allow their identification since some of them refer to flower traits, which become lost when cutting the flower peduncle (quiote) during the agave management and harvesting activities; therefore, it is necessary to define vegetative traits that can serve in the delimitation of species and varieties of this genus used in the production of mezcal (Figuero-Urbina *et al.*, 2017).

In the Northern Region of Guerrero, some agave species are mainly used for mezcal production, an activity that has promoted a high demand in plantations and wild populations (Delgado-Lemus *et al.*, 2014). Nevertheless, the knowledge that farmers have to identify the species and varieties of agave used for the production of mezcal is still limited, and they use local names (wide maguey or thin maguey), which can change depending on the place or region where they are used; therefore, not knowing the scientific name of these species limits their access to available information on forms of reproduction, management, and byproducts of these species and varieties, as well as to the certification processes of agave plantations and mezcal production.

Morphometric studies are used to identify patterns of variation in plant populations and to distinguish taxa within plant groups (Gonzalo *et al.*, 2012). Multivariate analyses have been used to analyze morphological variability and contribute to solving taxonomic problems in some complexes and species of the family *Asparagaceae* (Gutiérrez *et al.*, 2017). Clustering and ordering analyses, such as principal component analysis (PCA) and linear discriminant analysis (LDA), have made it possible to differentiate, separate, and identify wild and domesticated taxa of economic importance (Figuero-Urbina *et al.*, 2021; Barrientos-Rivera *et al.*, 2019; Arzaba-Villalba *et al.*, 2023).

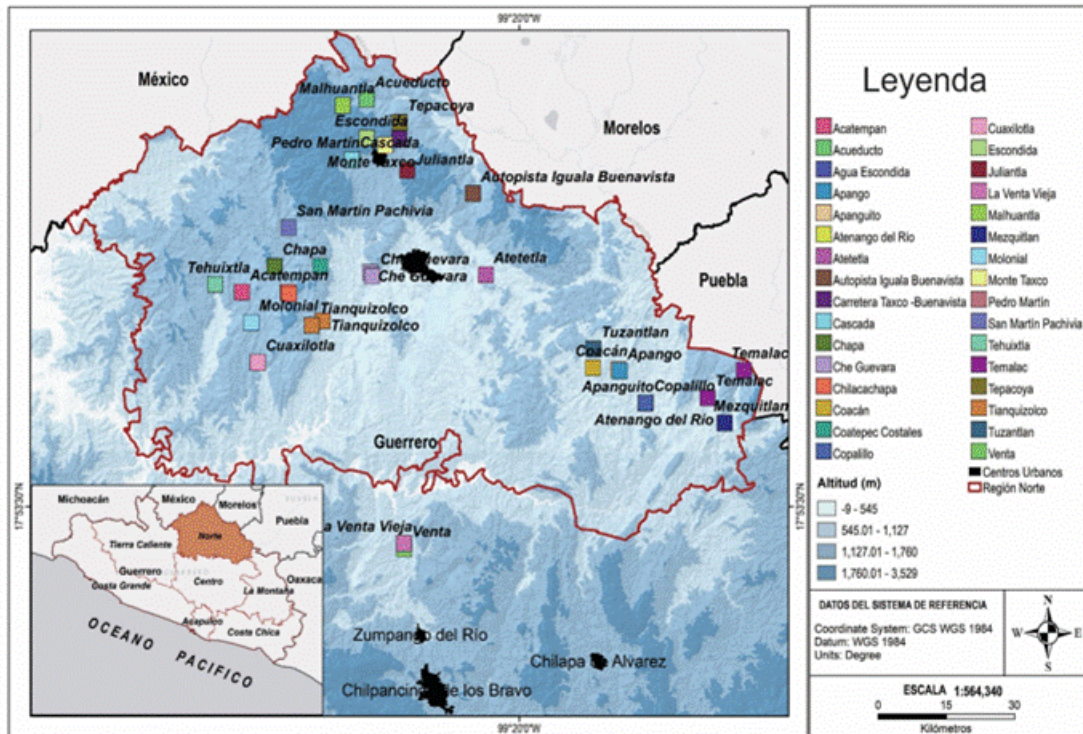
In this context, the objectives of this study were to characterize the morphometry of mezcal agaves from the Northern Region of Guerrero through two multivariate analyses to contribute to their taxonomic delimitation and identify morphometric traits that can be used by mezcal agave producers for the identification of these species.

Materials and methods

Location of the study area

The research was conducted in 31 localities in the Northern Region of Guerrero (Figure 1). Forty-five field trips were carried out from August 2016 to March 2023, where 230 specimens of nine *Agave* taxa were collected. The specimens were identified through the descriptions of Gentry (1982); García-Mendoza (2011).

Figure 1. Geographical location of the study locations.



Morphometric data measurement

Twenty-three morphometric traits (14 quantitative and nine qualitative) and one taxonomic trait were selected. The principal component analysis used 23 traits (Table 1) and the linear discriminant analysis employed 14 (Table 2). The species and varieties collected depended on the particular conditions presented by each specimen and sampling site. Quantitative traits were measured with the software of Image J version 2023.

Table 1. Factor loads of the principal components.

Traits	PC1	PC2	PC3	PC4	PC5
Rosette height (ALRos)	0.065	0.392	0.039	0.026	0.049
Rosette diameter (DmRos)	0.083	0.35	0.055	0.148	0.094
Number of leaves per plant (NHjs)	0.061	0.193	-0.335	-0.206	-0.605
Leaf length cm (LnHjs)	0.108	0.385	0.078	0.028	0.172
Width at the leaf base (AnBsHjs)	-0.211	0.276	0.149	-0.003	0.094
Wider leaf width (AnMyrHjs)	-0.272	0.185	0.132	-0.051	0.011
Distance from the base to the	0.1	0.354	0.086	0.152	0.1

Traits	PC1	PC2	PC3	PC4	PC5
widest part of the leaf (DtBsAnchHj)					
Number of teeth on one side of the leaf (NDts)	0.115	0.311	-0.267	0.166	0.187
Length of teeth in the middle of the leaf (LnDts)	-0.297	-0.018	0.066	0.113	-0.073
Width of teeth in the middle of the leaf (AnDts)	-0.290	0.04	0.061	0.058	-0.136
Distance between teeth in the middle of the leaf (DtEntrDts)	0.033	0.08	0.455	-0.437	-0.1
Terminal spine length (LrET)	-0.219	0.044	0.241	0.166	-0.219
Terminal spine width (AnET)	-0.087	0.029	0.107	0.64	-0.409
Distance from the terminal spine to the nearest tooth (DtETDte)	0.004	0.167	0.461	-0.064	-0.157
Leaf shape (HFrm)	-0.257	-0.047	-0.154	0.132	-0.188
Leaf margin (HMrgn)	-0.306	0.052	0.078	0.009	0.199
Glaucosity (Glaus)	0.126	0.25	-0.069	-0.05	-0.265
Leaf color (HClr)	0.148	-0.211	0.408	-0.018	-0.077
Side tooth shape (DLFrm)	-0.227	0.171	-0.141	-0.352	-0.113
Tooth uniformity (Dunfr)	-0.316	-0.065	0.044	0.023	0.072
Side tooth color (DLClr)	-0.292	-0.034	-0.067	0.118	0.309
Terminal spine shape (ETFrm)	-0.288	0.14	-0.163	-0.19	0.011
Terminal spine color (ETClr)	-0.294	0.011	-0.086	-0.193	-0.019
Standard deviation	3.031	2.211	1.373	1.134	1.025
Proportion of variance	39.95%	21.26%	8.21%	5.60%	4.57%
Cumulative proportion	39.95%	61.21%	69.42%	75.02%	79.59%

PC= principal component.

Table 2. Coefficients of the 14 traits in the three discriminant functions.

Traits	LD1	LD2	LD3
Rosette height	-0.14	0.47	-0.02
Rosette diameter	-0.16	-0.07	-0.11
Number of leaves per plant	-0.26	0.29	-1.07
Leaf length	-1.16	0.3	-0.04
Leaf base width	0.86	1.02	0.64

Traits	LD1	LD2	LD3
Wider leaf width	2.08	0.63	-0.16
Distance from the base to widest part of the leaf	0.12	-0.15	-0.02
Number of teeth on one side of the leaf	0.12	0.38	-0.27
Length of teeth in the middle of the leaf	0.66	-0.73	-0.42
Width of teeth in the middle of the leaf	0.31	-0.31	-0.42
Distance between teeth in the middle of the leaf	-0.29	0.32	0.18
Terminal spine length	0.61	-0.18	-0.01
Terminal spine width	-0.56	-0.13	-0.03
Distance from the terminal spine to the nearest tooth	0.11	0.08	0.46

LD= linear discriminant.

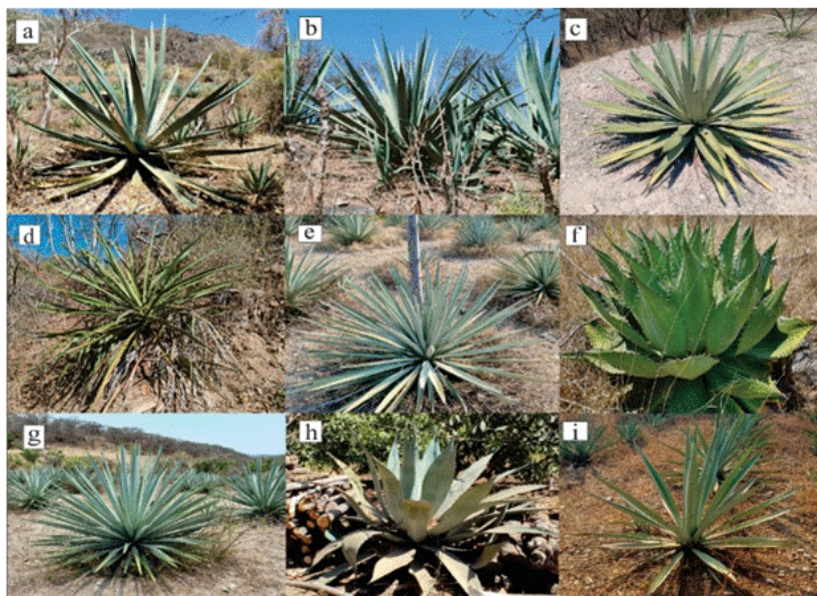
The traits and their data were systematized in a Microsoft Excel® database with each taxon identified. The two statistical analyses used a correlation matrix, the most important traits were selected, and then the principal component analysis (PCA) was performed with the princomp function of the stats package and the linear discriminant analysis (LDA) with the greedy.wilks function of the klaR package (Weihs *et al.*, 2005) using the R software version 4.0.3 (R Core Team, 2020).

Results and discussion

In the present study, 230 specimens corresponding to nine taxa were identified: *Agave americana* L. var. *americana* (four specimens), *A. americana* var. *oaxacensis* Gentry (nine specimens), *A. angustifolia* Haw var. *angustifolia* Gentry (105 specimens), *A. angustifolia* Haw var. *rubescens* (Salm-Dyk) Gentry (41 specimens), *A. angustifolia* Haw 'Espadín' (11 specimens), *A. cupreata* Trel & Berger (24 specimens), *A. rhodacantha* Trel (26 specimens), *A. salmiana* Otto ex Salm (two specimens), and *A. tequilana* F.A.C. Weber (eight specimens) (Figure 2).



Figure 2. Specimens of agave collected in the study localities: a) *A. americana* var *americana* (San Martín Pachivía); b) *A. americana* var *oaxacensis* (Coatepec Costales); c) *A. angustifolia* var *angustifolia* (Coacán); d) *A. angustifolia* var *rubescens* (Coacán); e) *A. angustifolia* 'Espadín' (Coacán); f) *A. cupreata* (San Martín Pachivía); g) *A. rhodacantha* (Coacán); h) *A. salmiana* (San Martín Pachivía); i) *A. tequilana* (San Martín Pachivía).



This expands the number of species and varieties known for Guerrero, since Huerta-Zavala (2018) mentioned only six species that belong to the *angustifolia* complex, whereas Barrientos-Rivera *et al.* (2019) pointed out the use of *A. cupreata*, *A. angustifolia* 'Espadín', *A. angustifolia* 'Zacatoro' in eight localities in the Central and Northern regions of Guerrero.

Principal component analysis

The first five PCs explained 79.59% of the observed variation present in the 230 agave specimens analyzed (Table 1); other morphometric studies in agave have needed two to six PCs to explain more than 46% to 83% variation, using between 57 and 174 specimens (Mora-López *et al.*, 2011; Huerta-Zavala 2018; Barrientos-Rivera *et al.*, 2019; Vázquez-Pérez *et al.*, 2020; Figueredo-Urbina *et al.*, 2021; Arzaba-Villalba *et al.*, 2023).

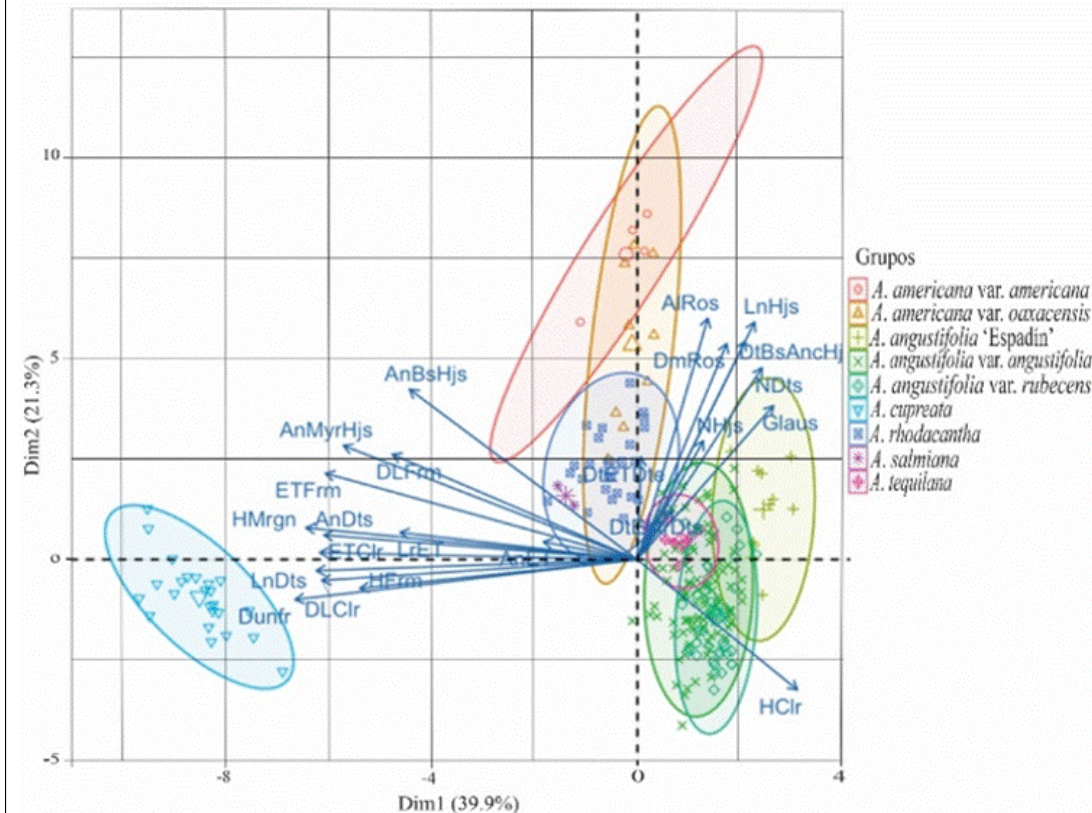
The first component contributed 39.95% of the total variance of the data; the traits that contributed the most (in order of their magnitude) are tooth uniformity, leaf margin, length of teeth in the middle of the leaf, terminal spine color, side tooth color, width of teeth in the middle of the leaf, terminal spine shape, wider leaf width, leaf shape, side tooth shape, terminal spine length, and width of the base of the leaf. All of the above traits have a negative correlation with PC1.

The second component explained 21.26% of the total variance; the characters that contributed the most (in order of their magnitude) are rosette height, leaf length, distance from the base to the widest part of the leaf, rosette diameter, number of teeth on one side of the leaf, leaf base width, glaucosity, and leaf color. These same characters have been used by other authors to separate Agave taxa (Mora-López *et al.*, 2011; Huerta-Zavala, 2018; Barrientos-Rivera *et al.*, 2019; Vázquez-Pérez *et al.*, 2020; Figueredo-Urbina *et al.*, 2021).

It is important to mention that only the graphic results of PCs 1 and 2 are presented because the simultaneous graphic representation of the 5 PCs is not possible. Considering the first two principal components, which accumulate 61.21% of all variance, the dispersion and morphometric trends of the taxa examined in the coordinate system defined by the first two PCs are observed

(Figure 3). In the bottom left, it was observed that the specimens of *A. cupreata* form a group with a clear separation from the other taxa, traits such as tooth uniformity and leaf margin provided more information in the separation of these specimens from the other groups.

Figure 3. Contribution of the studied traits to the first two PCs and morphometric trends of the taxa.



At the opposite part of the graph were the two specimens of *A. salmiana*, which had few specimens and did not form a group. However, an overlap was observed with the specimens of *A. americana* var. *oaxacensis* and *A. rhodacantha* since they share very similar measurements in rosette height and leaf base width, with *A. rhodacantha* differing from these taxa by presenting more than 100 leaves per specimen (Huerta-Zavala 2018).

In the top right (Figure 3), it is observed that the specimens of *A. americana* var. *americana* show an overlap with the specimens of *A. americana* var. *oaxacensis*, in addition to being separated from the rest of the taxa, with the specimens of this species forming a group mainly by higher values in the height and diameter of the rosette and in leaf length.

On the other hand, in the bottom and top right, various groups (taxa) that correspond to the *angustifolia* complex were observed (Rivera-Lugo *et al.*, 2018), among these were the specimens of *angustifolia* var. *angustifolia* and *angustifolia* var. *rubescens*, which were characterized by having small and thin lanceolate leaves in the var. *angustifolia* and in *A. tequilana* and linear in the var. *rubescens*, whereas the specimens of *A. angustifolia* 'Espadín' tend to cluster in the top right mainly because of uniformity in the number of teeth and glaucosity in the leaf (Figure 3).



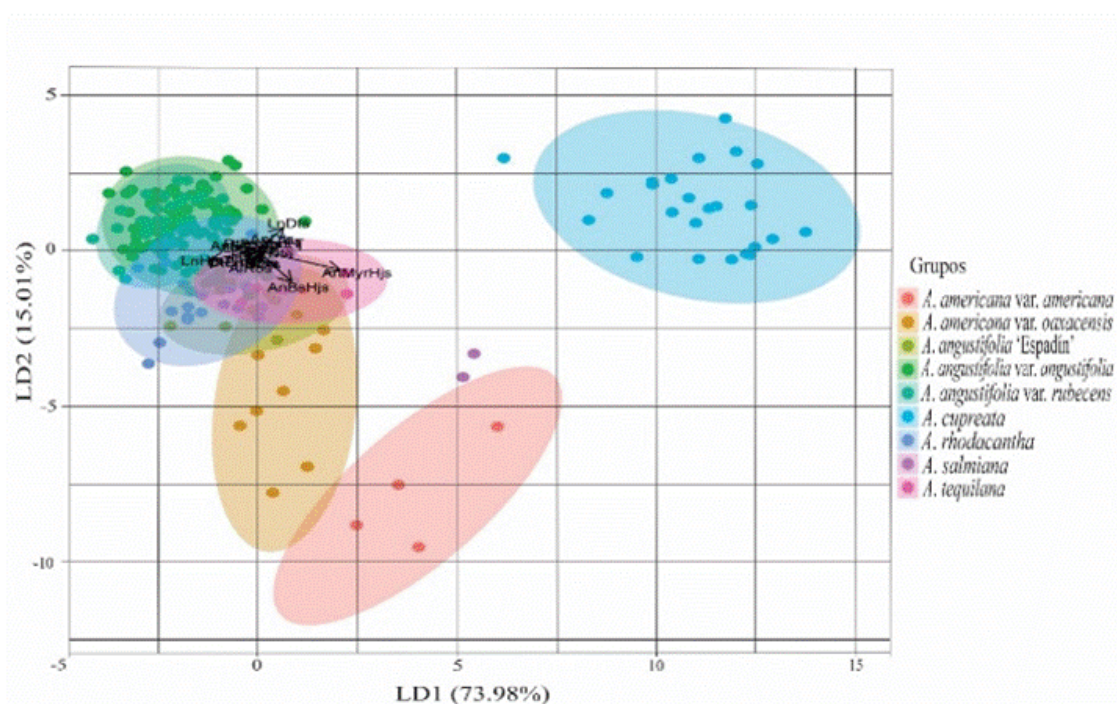
Linear discriminant analysis

Linear discriminant analysis with 14 traits correctly predicted the corresponding taxon in 86.5% of the specimens. The first linear discriminant function explained 74% of the variation in the data and the second linear discriminant function explained 15% and together they explained 89%.

In the first linear discriminant function, the traits that contributed the most were wider leaf width, leaf length, leaf base width, and length of teeth in the middle of the leaf. In the second linear discriminant function, the traits that contributed the most were leaf base width, length of the teeth in the middle of the leaf, wider leaf width, and number of teeth on one side of the leaf (Table 2); these traits have also been used by other authors to discriminate groups in agave taxa (Huerta-Zavala, 2018; Rivera-Lugo *et al.*, 2018; Figueredo-Urbina *et al.*, 2021).

In the top right (Figure 4), the specimens of *A. cupreata* can be seen, which differ from the other taxa. In the center are the two specimens of *A. salmiana*, which differ from the other taxa without constituting a group due to the small number of specimens. At the bottom of the graph are the specimens of *A. americana* var. *americana*, separating themselves from the other taxa, a situation very similar to *A. americana* var. *oaxacensis*, presenting a slight overlap with the specimens of *A. tequilana*.

Figure 4. Distribution of taxa with respect to the first two linear discriminant functions.



Finally, the specimens of *A. angustifolia* var. *angustifolia*, *A. angustifolia* var. *rubescens*, *A. tequilana*, *A. angustifolia* 'Espadín', and *A. rhodacantha* show an overlap between groups, which is understandable if one considers the phenotypic plasticity presented by the species and varieties of the *Angustifolia* complex to which all these taxa belong (Figure 4).

The LDA made it possible to clearly differentiate the specimens of *A. cupreata*, as well as the groupings of the specimens of *A. americana* var. *americana* and *A. americana* var. *oaxacensis*, which belong to the same species, differing mainly from each other by the number of leaves and wider leaf width, these two traits being lower in *A. americana* var. *oaxacensis*, which is similar to what was reported by Gentry (1982); García-Mendoza (2011).

On the other hand, wild specimens of *A. angustifolia* var. *angustifolia* and *A. angustifolia* var. *rubescens* belong to the same species, showing similarities with specimens from plantations of *A. tequilana*, *A. angustifolia* 'Espadín', which are considered members of this species, but already under humanization processes; therefore, Gentry (1982) treated them as different taxa. Rivera-Lugo *et al.* (2018) mentioned the genetic closeness between specimens of *A. tequilana* and *A. angustifolia* 'Espadín', stating that the latter taxon has a close relationship with *A. tequilana* and *A. rhodacantha*, which also belongs to the group of species that make up the *angustifolia* complex, so they share many similar characteristics with *A. angustifolia*.

When comparing the PCA and the LDA carried out in the present research, it was observed that the LDA better separates the specimens of the nine taxa studied, with only two discriminant functions, results that are similar to those mentioned by other authors (Huerta-Zavala, 2018; Rivera-Lugo *et al.*, 2018; Vázquez-Pérez *et al.*, 2020).

Barrientos-Rivera *et al.* (2019) carried out a morphological study of the 'maguey sacatoro' (*A. angustifolia*) in the state of Guerrero, pointing out that the taxa of *A. angustifolia* 'sacatoro' and *A. angustifolia* 'espadín' are closely related due to the selection and vegetative propagation that they have received for the production of mezcal; in addition to the fact that cultivated taxa differ from wild populations due to the domestication process that have received; information that is very similar to that reported by Rivera-Lugo *et al.* (2018) in their study on the morphological and genetic variation of the *A. angustifolia* complex, mentioning that the phenotypic dispersal of these taxa is related to the origin (wild or cultivated) and the management given to the plant.

With the present research, the known distribution area of *A. rhodacantha* in Guerrero was expanded to six localities since, previously, Huerta-Zavala (2018) reported its existence in only four localities. *Agave rhodacantha* was only known from the states of Sonora, Sinaloa, Nayarit, Jalisco, Durango, and Oaxaca (Gentry, 1982; Rivera-Lugo *et al.*, 2018). *A. angustifolia* var. *angustifolia* is the most frequent taxon in producers' plots, whereas *A. salmiana* is the scarcest, with only two specimens collected.

As in other research, *A. angustifolia* var. *rubescens* is only found in wild form and is only used for mezcal production when there is a shortage of agave in the localities (Huerta-Zavala, 2018). In this context, it is important for producers to manage and conserve the diversity of *Agave* taxa used for mezcal production since, depending on the species or variety of *Agave*, it can take between seven, ten, or thirty-six years or more to reach harvest maturity (Alducin-Martínez *et al.*, 2023).

On the other hand, given the increase in the demand for mezcal, at the regional level, the introduction of species from other regions of the country is observed, such as *A. angustifolia* 'Espadín' (Oaxacan Espadín) and *A. tequilana*, so it is important to analyze the environmental and economic impacts that the introduction of these species may generate.

Conclusions

The knowledge was expanded to nine species and varieties of agave used for the production of mezcal in the Northern Region of Guerrero. These can be differentiated from the traits identified by the LDA, which are wider leaf width, leaf length, leaf base width, and length of the teeth in the middle of the leaf. This analysis turned out to be more suitable for their identification.

The identification of the species and varieties of agave used by producers in the production of mezcal will allow them to access information on ways of propagation, management, and improvement of productive yields and will facilitate the certification processes of their agave plots.

Differentiating and knowing the scientific name of the agaves used for the production of mezcal will allow producers to give added value to their product since providing more information to consumers on the label of the bottled product can be an element that provides them with security and confidence. Therefore, it is essential to promote the diversity of native agave species.

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