RESEARCH ARTICLE

The genetic diversity of wild grapes in Mexico

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Abstract This is the first report evaluating the genetic diversity of Mexican grape species utilizing DNA-based markers to understand the distribution of grape species, and patterns of hybridization. The study utilized accessions maintained in three collections in Mexico, one in the USA and recently collected germplasm. Fifteen SSR markers were used to develop a fingerprint database to identify unique germplasm. Two different clustering analyses without prior population assignment, were used to identify groups that were verified by a Discriminant Analysis of Principal Components and a Principal Coordinate Analysis. Genetic diversity estimates were made across and within groups to validate the results obtained from the clustering analyses. Multiple clustering analyses and diversity parameters supported six genetic groups representing different geographic regions. The Northeastern group was the most diverse with a geographic range extending to Eastern and Central Mexico, while the Coahuila group was the least diverse. *Vitis arizonica* Engelm. and *Vitis cinerea* Engelm. ex Millardet were the most abundant species with many hybrid forms. We provide evidence that wild grape species in Mexico follow the physical barriers of mountain ranges like the Sierra Madre Oriental with an east–west divide and the Trans-Mexican Volcanic Belt as a corridor for gene flow among different grape species. Additional collections are required to fully understand the extent of hybridization and to clarify hybrid zones.

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Introduction

Mexico possesses a rich floristic diversity primarily because it is in the transition zone between the Nearctic and the Neotropical biogeographical regions. This diversity also originates from Mexico's unique geographical location between the Pacific and Atlantic oceans and the presence of large and evolving

mountain systems and deserts (Vanderplank et al. 2018). Mexico is part of the group of countries that hold 70% of the world's biodiversity (Vanderplank et al. 2018), and ranked fourth behind Brazil, China and Colombia (Villaseñor 2016). Central Mexico, where the Nearctic and the Neotropical regions overlap, represents a complex ecological area with physical barriers and different microclimates that range from warm and humid areas to cold highlands that house a wide diversity of biotas which interact and evolve within their unique niches (Padilla et al. 2014). It has been previously reported that mountains on the Trans-Mexican Volcanic Belt act as a physical barrier and provide a separation between the north and south biota (Mastretta-Yanes et al. 2015). However, results from other studies indicate an east–west pattern, with Tamaulipas, Yucatan and the Mexican Gulf as determinants of the eastern clade and the remaining provinces defining the western clade (Corral-Rosas and Morrone 2017).

Wild grapevines have had a unique cultural significance for the indigenous groups of Mexico for thousands of years. The Aztecs named the grapevine berry "*acacholli*" and likely maintained wild vines as an important source of food and medicine (Franco-Mora and Cruz-Castillo 2012). Fruit from the wild grapevines was harvested to make juice, raisins and jelly. The leaves were used for industrial and medicinal purposes to treat skin eczema, swollen eyes, fevers, as appetite stimulants and to treat athlete's foot (CONAFRUT 1973). More recently, there has been increasing interest in harvesting grape seed oil and meal from wild grapevines (Juárez-Trujillo et al. 2017) and phenolic compounds for pharmacological use from leaves (Tobar-Reyes et al. 2011). Studies have also shown that resveratrol, an antioxidant compound with great pharmacological potential, can be extracted from grape leaves. Producers of artisan alcoholic beverages, are using endemic wild grape species to produce semisweet wine (Galindo-Tovar et al. 2019).

It is believed that about 30 *Vitis* species exist in North America and many of them have a wide geographic range that extends to southeastern Mexico and Central America (Heinitz et al. 2019; Walker et al. 2019). Very little is known about the current genetic diversity of wild grape germplasm in Mexico. Wild grapevines have been identified mostly in temperate climates and a few have been found in subtropical areas (Rzedowski and Calderón de Rzedowski 2005). The grape germplasm collections in the United States also have a limited representation of wild grapes from Mexico and Central America. Most of the germplasm was collected in 1961 by H.P. Olmo (University of California, Davis) and later by B.L. Comeaux in the late 1980s and early 1990s, who described wide variation in grape germplasm (Comeaux 1987). Genetic studies have shown that wild germplasm from Mexico is a valuable resource for resistance to Pierce's disease, and a wide range of nematodes (Riaz et al. 2007, 2018b; Xu et al. 2008). Changes in land use, the expansion of urban areas, animal grazing, altered water rights and uses have led to deforestation, and great reductions in natural habitat and erosion of genetic diversity (Sabás-Chavez et al. 2018). The *Vitis* species of Mexico represent a diverse and inadequately studied germplasm resource, whose conservation is important for grape breeding, cultural heritage studies and understanding of their role in the ecosystem.

In 2005, the Mexican national program "*Red de Vid*" was financed by the Secretary of Agriculture and Rural Development (SAGARPA) and aimed at collecting, describing and preserving wild grapevines in Mexico (Franco-Mora et al. 2012). A national germplasm bank was established at the *Salvador Sánchez Colín Center of Scientific and Technologic Investigation* (CICTAMEX) in Coatepec-Harinas, State of Mexico (Sabás-Chavez et al. 2018). According to studies done by "*Red de Vid*", wild grapevines in Mexico have been identified in four main climates: temperate, warm, semidry and dry (Franco-Mora and Cruz-Castillo 2012). These collections have been used for studies on taxonomy, floral biology, medicinal compounds, agricultural and culinary uses. Most of the studies that evaluate the diversity of collections of wild grapevines rely on leaf and fruit morphology to divide them into different groups and use ampelographic features to determine species nomenclature (Comeaux 1991; Cruz-Castillo et al. 2009; Franco-Mora and Cruz-Castillo 2012; Franco-Mora et al. 2008a, 2008b).

Experience is necessary to reliably identify grape species based on morphological features, since they often vary within different climates, plant age, and growing conditions. Moreover, it is very hard to identify hybrid forms and designate them into correct species. On the other hand, DNA-based molecular

markers are a robust and valuable tool capable of providing reliable results. The international grape research community has employed simple sequence repeats (SSRs) markers to maintain grape germplasm collections (Dangl et al. 2015; Laucou et al. 2011), determine parent progeny relationships (Bowers et al. 1999; Lacombe et al. 2013; Riaz et al. 2019), identify grape cultivars, to assess germplasm diversity (Riaz et al. 2013, 2019; This et al. 2004; Vouillamoz et al. 2006), develop genetic maps (Doligez et al. 2006; Pap et al. 2016; Riaz et al. 2004, 2012) and for use in marker-aided selection in grape breeding programs (Riaz et al. 2008). In this study, we employed SSR markers to develop a fingerprint database of wild germplasm collected from Northern and Central Mexico. Germplasm was acquired from three Mexican germplasm collections and wild vines sampled from their native habitat. Grape germplasm collected from Mexico in 1961 and 1990, and now maintained at the National Clonal Germplasm Repository, USDA-ARS, Davis (NCGR-Davis), was also included. The main objectives of the study were to: (a) develop fingerprint profiles of wild *Vitis* to aid germplasm repositories in the identification of unique landraces, wild species and hybrids, duplicates and other propagation mistakes; (b) to gain better understanding of *Vitis* genetic diversity with emphasis on species grouping and hybridization patterns; and (c) to identify ecological and geographic distribution trends to aid future germplasm collection efforts.

Materials and methods

Plant material

A total of 317 Mexican accessions were utilized in this study (Table 1). Leaf tissue for 206 accessions was acquired from the germplasm banks at the Zumpahua-can (maintained by the Autonomous University of the State of Mexico, Toluca (UAEMex)), CICTAMEX maintained at Coatepec-Harinas with support from the National System of Phytogenic Resources (SINAR-EFI), and Universidad Autonoma de Chihuahua (UACh). Two collection trips were taken to collect 45 additional accessions from Nayarit, and Coahuila states (Table 1). Additionally, a subset of 66 accessions collected from Mexico by H.P. Olmo in 1961 (b series), and by B.L. Comeaux in the early 1990s

(DVIT series) and maintained at the NCGR-Davis were added to the study to analyze their relationship to the set of recently collected material. Table 1 presents the collection sites and the germplasm repositories, and Suppl. Table 1 in the Online Resources provides the collection details of each sample used in this study.

GPS coordinates and habitat notes were recorded when the samples were collected from their natural habitats. Most of the accessions were phenotyped for leaf morphology, shoot tip appearance and possible species identity based on their ampelographic features. A fraction of the accessions was also photo documented to aid with species identification (Supplementary material) Young leaves were collected from actively growing shoots, placed between layers of filter paper, air-dried and stored in paper envelopes for further use.

Genotyping

Five grams of dried leaf tissue was used to extract DNA following a modified CTAB protocol previously described (Riaz et al. 2008). A set of 20 SSR markers were selected that included six standard markers recommended by the grape research community and nine other markers that have been used for previous genotyping studies (Bowers et al. 1999; Dangl et al. 2015; Doligez et al. 2006; Riaz et al. 2020). Polymerase Chain Reactions (PCR) were performed using fluorescent labeled primers following the protocols of an earlier study (Riaz et al. 2018a). The PCR products were separated using an ABI 3500 capillary electrophoresis analyzer with GeneScan-500 Liz Size Standard (Life Technologies, Carlsbad, CA). GeneMapper 4.1 software (Applied Biosystems Co., Ltd., USA) was used to analyze and size the fragments. A set of six control samples were used to standardize fragment size between runs. The genotypic data from the 66 accessions maintained at the NCGR-Davis was acquired from other study sets in the Walker lab (Riaz et al. 2018b, 2020).

Data analysis

Data quality control

Accessions with more than 40% missing genotypic data and SSR markers with more than 15% missing data were omitted from the analysis. The *poppr*

Table 1 Summary of the germplasm collection sites used in this study

Collection sites	No. of accessions	No. of unique accessions	Used for analysis	Putative species in background	Collection location
Zumpahuacan germplasm collection	39	26	25	*V. cinerea, V. biformis, V. bloodworthiana*	Zumpahuacan, State of Mexico
CICTAMEX germplasm collection	107	77	66	*V. cinerea, V. tiliifolia,V. bloodworthiana*	Coatepec-Harinas, State of Mexico
UACh germplasm collection	60	60	59	*V. arizonica*	Chihuahua, Chihuahua
NCGR-Davis germplasm collection	66	66	66	*V. nesbittiana, V. cinerea, V. tiliifolia, V. arizonica, V. biformis*	Davis, California
Coahuila collection trip	16	16	16	*V. arizonica*	Parras de la Fuente, Coahuila
Nayarit collection trip	29	15	15	*V. cinerea, V. bloodworthiana*	Tepic, Nayarit
Total	317	260	247		

package in R (Kamvar et al. 2014; R Core Team 2019) was used to construct a genotype accumulation curve to identify the sufficient number of SSR markers to distinguish duplicates. Accessions with duplicates were identified by using the *clonecorrect* function and discarded from future analyses. A total of 247 unique accessions with genotypic data for 15 SSR markers were moved to the next round of analysis (Table 1).

The objective of the first round of analysis was to identify and remove feral *Vitis vinifera* L. hybrids, pure *V. vinifera* cultivars, and commercially used rootstocks in the collected material. The SSR marker data of 161 accessions that represented commercial *V. vinifera* varieties and rootstocks from an earlier study in the Walker lab was obtained (Riaz et al. 2019) and used as a reference to identify accessions that could disrupt the analysis. This data set also included well-known grape species (*Vitis berlandieri* Planch., *V. riparia* Michx., *Vitis rupestris* Scheele and their hybrids) native from the US to identify a possible species affinity to Mexican germplasm. DARwin software V5.0.158 (Perrier and Jacquemoud-Collet 2006) was used to build a Neighbor Joining Unweighted dendrogram from a composite set of 408 accessions. After the identification of *V. vinifera* cultivars and potential hybrids, the 161 reference accessions were excluded from further analysis.

Population structure

STRUCTURE V2.3.1 (Pritchard and Falush 2007) was used to determine the adequate number of genetic groups (K) without prior population designation. Simulations were run assuming an admixture model, 100,000 Markov Chain Monte Carlo (MCMC) iterations with a burn-in of 50,000 and a maximum of 20 K, with 20 runs per K. The STRUCTURE results were processed using Structure Harvester (Earl 2012) to find the most likely K value based on the estimated Ln probability (ln Pr(X/K)), and CLUMPPAK (Kopelman et al. 2015) to obtain the probability assignment of each genotype to different clusters. Structure plot V2 (Ramasamy et al. 2014) was used to plot the results in order of Q-values.

A Principal Coordinates Analysis (PCoA) was carried out with DARwin. Additionally, an Unweighted Pair Group Method with Arithmetic mean (UPGMA) fan dendrogram was calculated based on Bruvo's distance with 50 bootstraps performed, using the *poppr* package. A Discriminant Analysis of Principal Components (DAPC) was used to observe differences among the a priori found STRUCTURE groups (Jombart et al. 2010) using the *adeneget* package in R.

A map of Mexico's biogeographic provinces (Morrone et al. 2017) was built in ArcGIS (ESRI

2011) by using the shapefiles developed by Morrone and collaborators (Morrone et al. 2017). The accessions' GPS information and corresponding Q-values assignments from the STRUCTURE results were plotted on the map.

Genetic diversity assessment

GenAlEx 6.5 software (Peakall and Smouse 2006) was used to evaluate the following genetic diversity parameters: allele frequency by population, number of alleles (Na), number of effective alleles (Ne), Shannon's Information Index (I), observed heterozygosity (Ho), expected heterozygosity (He), Fixation Index (F), estimates of Pairwise Nei's Genetic Distance, estimates of pairwise population (Fst) and the deviation of Hardy–Weinberg equilibrium (HWE). Evenness was calculated for every population using the *poppr* package in R.

Results

Data quality check for duplicates and V. vinifera hybrids

In this study, 317 accessions were acquired from different germplasm repositories and collection trips in Mexico. The genotype accumulation curve (Supplementary Fig. 1; see Online Resources with this article) identified that only 8 to 9 SSR markers were sufficient to separate unique multi locus genotypes (MLGs), indicating that the 15 SSR markers used in this study are robust enough to identify duplicates and other misnaming errors within collections. The clone correction analysis identified 260 unique accessions within the study set, indicating that 57 accessions within two collections (the Zumpahuacan and the CICTAMEX) and half of the samples from the Nayarit collection trip were duplicates (Table 1). Collection records indicated that germplasm was exchanged from the private Zumpahuacan germplasm repository to CICTAMEX. A total of 77 accessions out of 107 tested samples from CICTAMEX were unique. Interestingly, 23 other accessions that had similar genotypic profile to *P-11* were identified for between the CICTAMEX and the Zumpahuacan germplasm repositories that were reportedly collected from different locations. Accession *P-11* and its many duplicates

were also identified as different grape species (*Vitis bloodworthiana* Comeaux, a *Vitis cinerea* Engelm. ex Millardet hybrid, and *Vitis tiliifolia* Humb. & Bonpl.). For the Zumpahuacan germplasm collection, 26 accessions were unique. Accessions that were duplicated and redundant between different collections are presented in Supplementary Table 2 in the Online Resources.

The Neighbor Joining Unweighted dendrogram from the preliminary analysis indicated no affinity of Mexican accessions to the samples of three grape species: *V. berlandieri*, *V. riparia* and *V. rupestris* (results not shown). No match with known rootstocks was found either. Three accessions (CH-05 from Chihuahua, RV-13 and RV-91 from Puebla) grouped with *V. vinifera* varieties, indicating that they were potential *vinifera* hybrids, and were excluded from further analysis to avoid skewed results. A final set of 247 unique accessions was moved forward for population analysis and diversity assessment.

Population structure and clustering analysis

The STRUCTURE program was used to determine the number of clusters within the data set of 247 accessions. The delta K value suggested two possibilities: K = 2 and K = 6 (Supplementary Fig. 2). It is well established through simulation studies that with a representative number of markers, the delta K method frequently identifies K = 2 as the top level of hierarchical structure (Janes et al. 2017). Therefore, K = 6 was adopted and it also had minimum variation between reps. Evanno's method provided a similar answer (Supplementary Table 3). The cumulative Q-values for K = 6 that reflect a proportion of a given individual's genome originating from a given population were obtained from the CLUMPPAK program. A Q-value threshold of 0.80 was selected to assign accessions to a particular genetic group. Supplementary Table 4 presents the Q-value assignments of 247 accessions to six groups. Accessions with Q-values below 0.80 were called admix. The UC Davis germplasm samples collected in 1961 by Olmo (b series) and Comeaux in the 1990s (DVIT series) had a higher degree of hybridization as indicated by their Q-values.

Figure 1 shows the bar plot for the six genetic groups that represent different regions: Eastern Mexico (red), Northeastern Mexico (purple), Coahuila
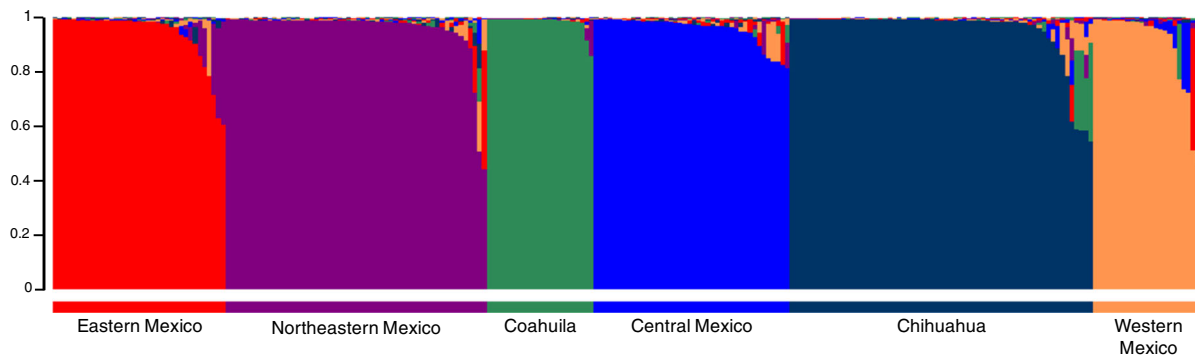
Fig. 1 Graphical representation of the inferred population structure for K = 6 using the model-based software STRUCTURE. Groups were named according to the geographic location: Eastern Mexico (red), Northeastern Mexico (purple), (green), Central Mexico (blue), Chihuahua (navy blue), and Western Mexico (gold). The same color scheme was used across all the presented figures. Figure 2 shows the collection location of accessions on the map of Mexico subdivided into fourteen Nearctic and Neotropical biogeographic regions with Coahuila (green), Central Mexico (blue), Chihuahua (navy blue), and Western Mexico (gold). Each vertical line represents one sample. Accessions were ordered from high to low Q-value in each group

the Q-value assignments for each accession obtained from the STRUCTURE program. The regions with most of the collected accessions were: the Northern and Southern Sierra Madre Occidental, Northern and Southern Sierra Madre Oriental, the border between the Balsas Basin province and the Trans-Mexican
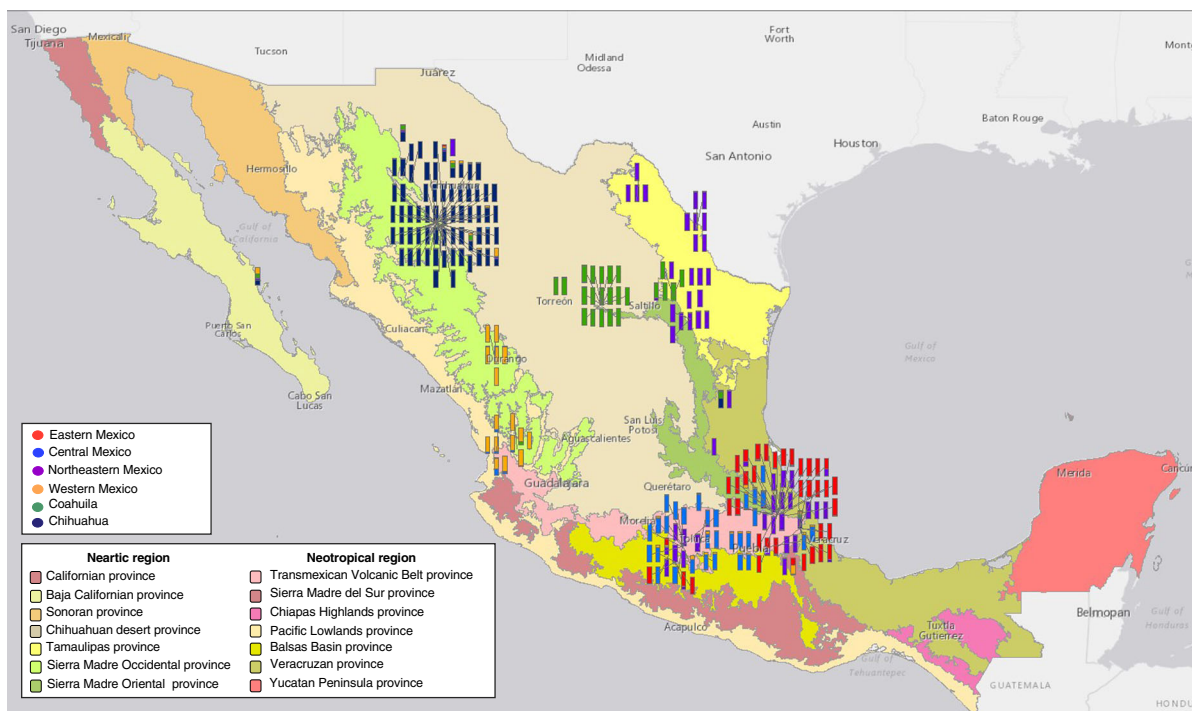
Fig. 2 Geographical distribution of accessions used in this study and their STRUCTURE results (K = 6). Every colored bar represents one accession and the proportion of Q in each of the six genetic groups. Accessions without reported GPS data were unable to be plotted on the map. Bar colors represent group assignment. Colors in the map represent biogeographical provinces obtained from previous studies

Volcanic Belt, the Veracruzan province, and the Tamaulipas Province (Fig. 2).

The East, Central and Northeastern Mexico groups consisted of 38, 42 and 55 accessions (Fig. 2, and Suppl. Table 4) respectively. The accessions from these three groups overlapped geographically. Specifically, the Northeastern Mexico group was spread between the Veracruzan province and central Mexico (Fig. 2) and extended to northern Mexico. Interestingly, the Northeastern group has the Sierra Madre Oriental mountain range as a physical barrier that separates it from western populations. The Sierra Madre Oriental is also a geographic continuum that leads to the Trans-Mexican Volcanic Belt, which crosses central Mexico horizontally, going from Cabo Corriente, Nayarit on the western coast, to the Sierra de Chinconquiaco, Veracruz on the eastern coast (Fig. 2). The groups resulted from the STRUCTURE clustering analysis also reflected the geographical barriers and continuum.

The Western Mexico group (gold) consisted of 24 accessions and six accessions were admixed with the Central Mexico population, suggesting the movement of genetic material in the Trans-Mexican Volcanic Belt. Interestingly, accession b42-26 from Loreto, Baja California Sur, has a unique genetic mix representing three different groups indicated by the Q-values. This accession has a 30% overlap with the Western Mexico group, a third with the Chihuahua group and a third with the Coahuila group (Supplementary Table 4). The genetic groups in northern Mexico—Chihuahua (navy blue) and Coahuila (green) –consisted of 65 and 23 accessions, respectively. Both groups were more isolated, particularly in comparison to the Northeastern group (purple) that was widely distributed across Mexico.

Three additional clustering analyses were employed to verify the groups obtained from the STRUCTURE analysis. The UPGMA fan dendrogram based on Bruvo's distance matrix showed two outgroups and a main clade (Fig. 3). A small dendrogram was drawn for simplification to show the different clades clearly. Accessions from the Northeastern group (purple) and the Coahuila group (green) were outgroups and the major clade contained the remaining four groups. Within the major clade, two subgroups were found where Chihuahua (navy blue) was a separate branch, and the remaining groups also split with Central Mexico (blue), Eastern Mexico (red) and

Western Mexico (gold) branched together. Geographically, this dendrogram indicated an overlap of groups in the Trans-Mexican Volcanic Belt, and a separation between groups in the east and west. The UPGMA results were similar to the STRUCTURE analysis with few exceptions. Accession P-11 was placed in the Eastern group (red) instead Western Mexico group (yellow) in the UPGMA results. The STRUCTURE analysis found this accession was admix, with a Q-value of 0.448 for inclusion in the Eastern Mexico group. RV-96, another highly admix accession according to the STRUCTURE results, shared equal genetic affinity to the Eastern Mexico and Northeastern Mexico group (Suppl. Table 4). The misplaced accessions identified with the UPGMA results E-36, RV-58, b40-61, b40-14, and b40-13, were all admix with genetic affinity to different groups.

The PCoA analysis, which relies on the simple matching distance parameter, found a pattern similar to the one observed in the UPGMA dendrogram (Figs. 3 and 4). The two PCoA axes explained a total of 18.22% of the observed variance. Nineteen admix accessions (pink) identified by the STRUCTURE analysis were placed between different genetic groups (Fig. 4). The Chihuahua group separated from the other three groups along the upper quadrants, suggesting genetic dissimilarity with the Eastern, Western and Central Mexico groups. The Northeastern Mexico group (purple) was differentiated from other groups, whereas, marginal differentiation of accessions from the Eastern (red), Western (yellow) and Central Mexico (blue) groups was observed. The accessions in the Coahuila group made a sister group with the accessions from the Northeastern Mexico indicating that they are genetically similar.

A DAPC analysis that used *a-priori* information of groups identified in the STRUCTURE program was used to confirm the placement of accessions into different genetic groups (Fig. 5). During the first cross-validation DAPC analysis (Suppl. Figure 3), 40 principal components (PCs) had the highest proportions of successful outcome prediction (0.991667) and the lowest Mean Square Error (MSE) of 0.0263. To refine results, a second cross-validation analysis was conducted (Supplementary Fig. 4), resulting in a total of 36 PCs retained, with a cumulated variance of 73.7% (Fig. 5, top right). Five axes of the Discriminant Analysis (DA) were saved and two of them are shown (Fig. 5, top left). The Coahuila and Chihuahua
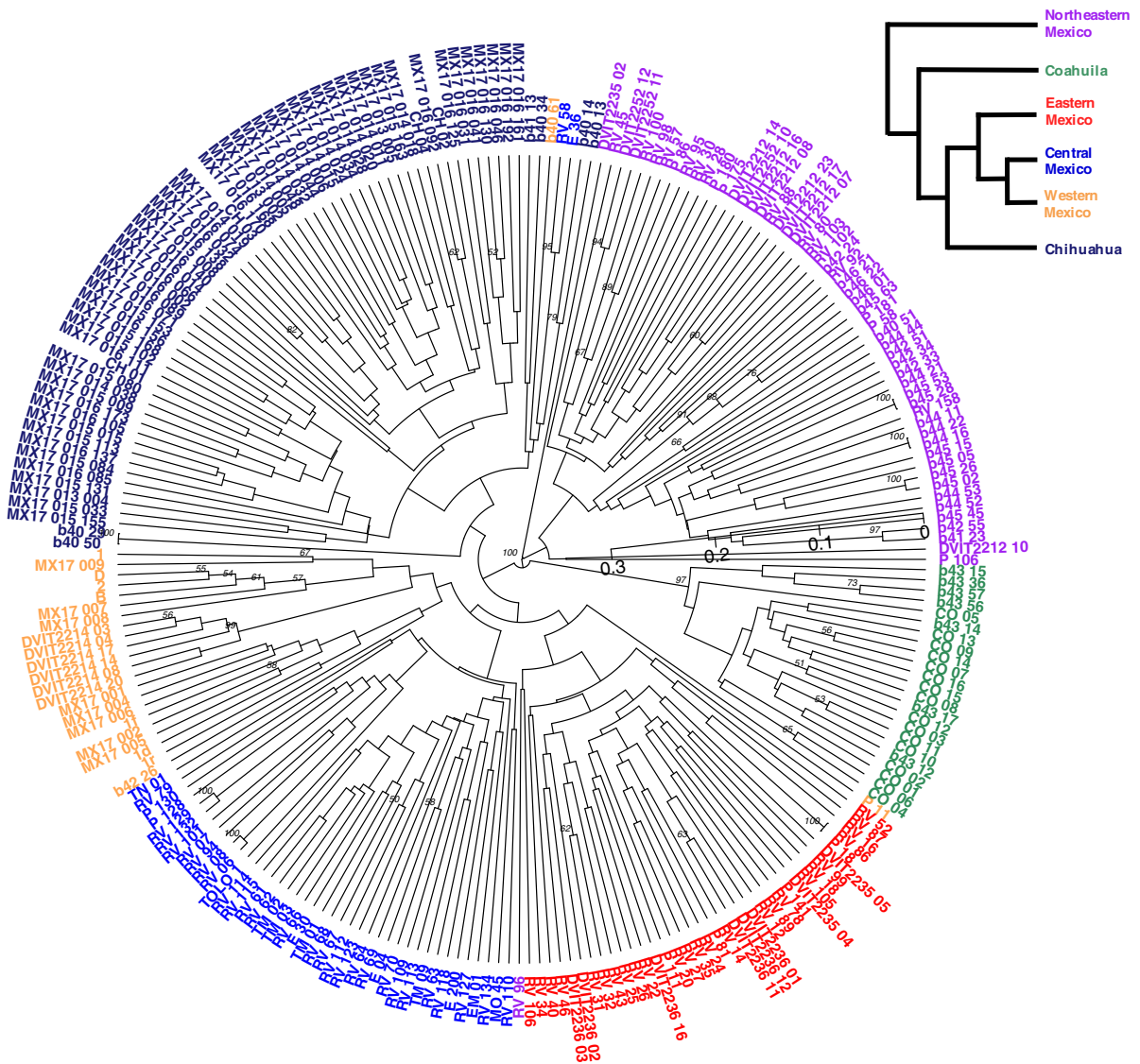
Fig. 3 UPGMA rooted dendrogram based on Bruvo's genetic distance from 15 SSR markers. Colors denote the group assignment previously obtained by STRUCTURE. Accessions placed outside of their color block are admix. Bootstrap values are indicated at the node. Red: Eastern Mexico; Purple: Northeastern Mexico; green: Coahuila; navy blue: Chihuahua; gold: Western Mexico. Upper right: simplified dendrogram. Branch length in the dendrogram does not depict any genetic distance

groups were clearly differentiated, as they did in the UPGMA and PCoA. Interestingly, the Coahuila group, in the lower quadrant, was the most differentiated from all groups, contrary to the results in the PCoA, where it made a sister group next to Northeastern accessions indicating genetic similarity. In contrast, the Northeastern and Eastern Mexico groups showed similar genetic variability, which is also different from the results in the PCoA. The Western and Central

Mexico genetic groups overlapped as they did in the PCoA. Overall, the results from the DAPC analysis were similar to the UPGMA and PCoA analyses: the Eastern and Northeastern groups that have no geographical barrier were closer to each other, the Central and Western Mexico groups that are connected by Trans-Mexican Volcanic Belt overlapped, and the Chihuahua and Coahuila groups that are physically
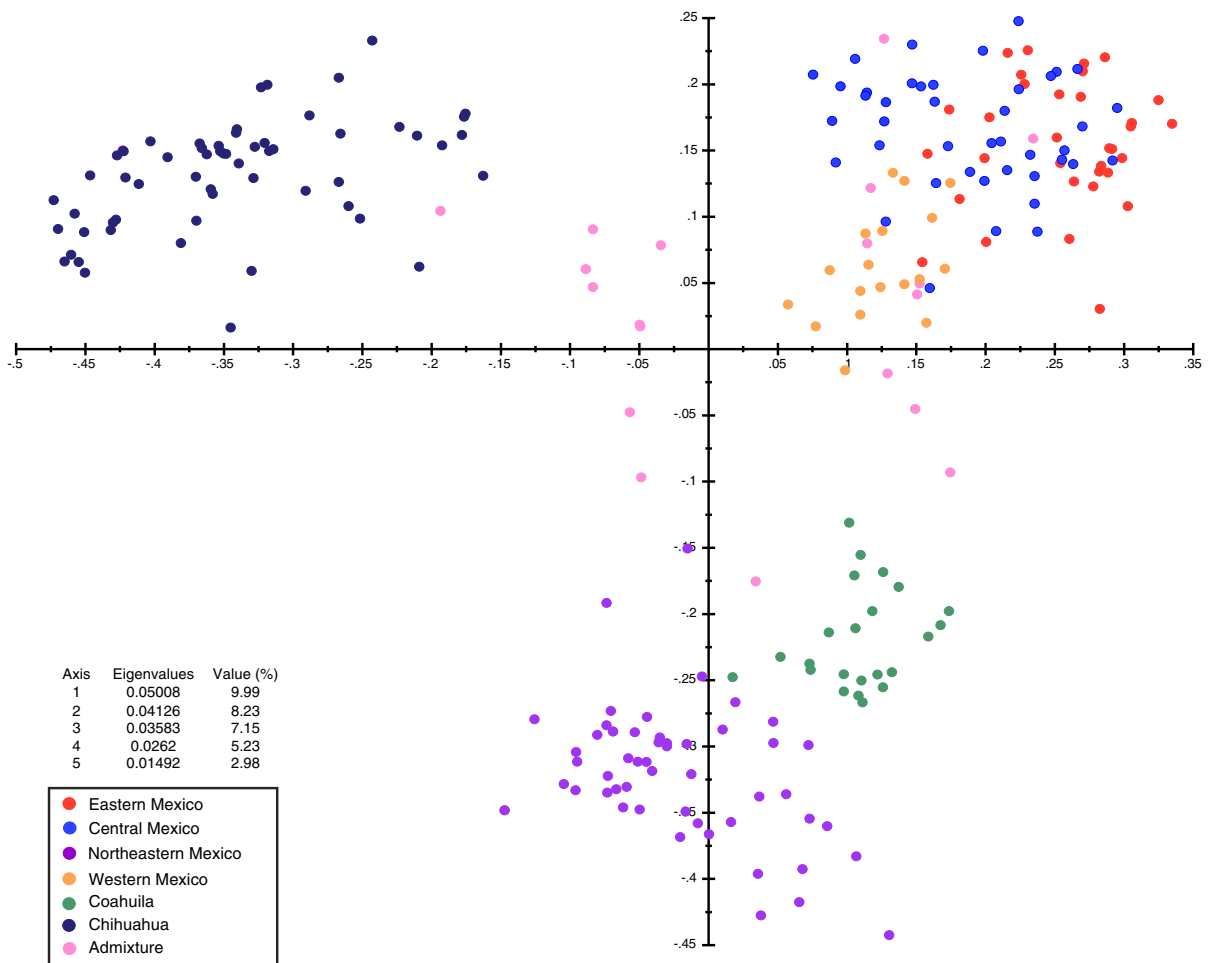
Fig. 4 Principal Coordinate Analysis (PCoA) of 247 accessions based on 15 SSR markers using DARWIN software. Accessions were labeled with the corresponding color for the group to which they belong. Eigenvalues for the four axes are shown below. Shown axes 1 and 2 explain 9.99 and 8.23% of the variation, respectively

separated by the Chihuahuan Desert were genetically very distinct.

Species composition of six genetic groups

Based on the general morphological features of leaves, and shoot tips when available, each group was given a possible species ID (Supplementary Table 1; see Online Resources). Table 2 presents a summary of putative species within each genetic group and their general morphological traits. Most of the Eastern Mexico group contained forms of *V. cinerea* and hybrids with visible *V. tiliifolia* characteristics. Similarly, accessions from Northeastern Mexico were also described as forms of *V. cinerea* based on the

morphological features. The Coahuila group consisted of different forms of *Vitis arizonica,* Engelm. with some that appeared to be *V. arizonica/cinerea* hybrids. The group from Central Mexico was recognized as *V. bloodworthiana* with influence from *V. cinerea.* The Chihuahua group consisted of accessions that were recognized as forms of *V. arizonica* with possible introgression from *Vitis candicans* Engelm. ex Durand. Western Mexico appeared to contain accessions that were recognized as *V. bloodworthiana/cinerea* hybrids (Suppl. Table 4).

Fig. 5 Discriminant Analysis of Principal Components. DAPC maximized the difference between genetic groups and minimized differences within individuals in the same group. A total of 36 Principal Component (PCs) (top right) were retained during the analysis with a cumulated variance of 73.7%. Two out of the five axes retained in the Discriminant Analysis (DA) are highlighted in black (top left) corresponding to the horizontal and vertical axes
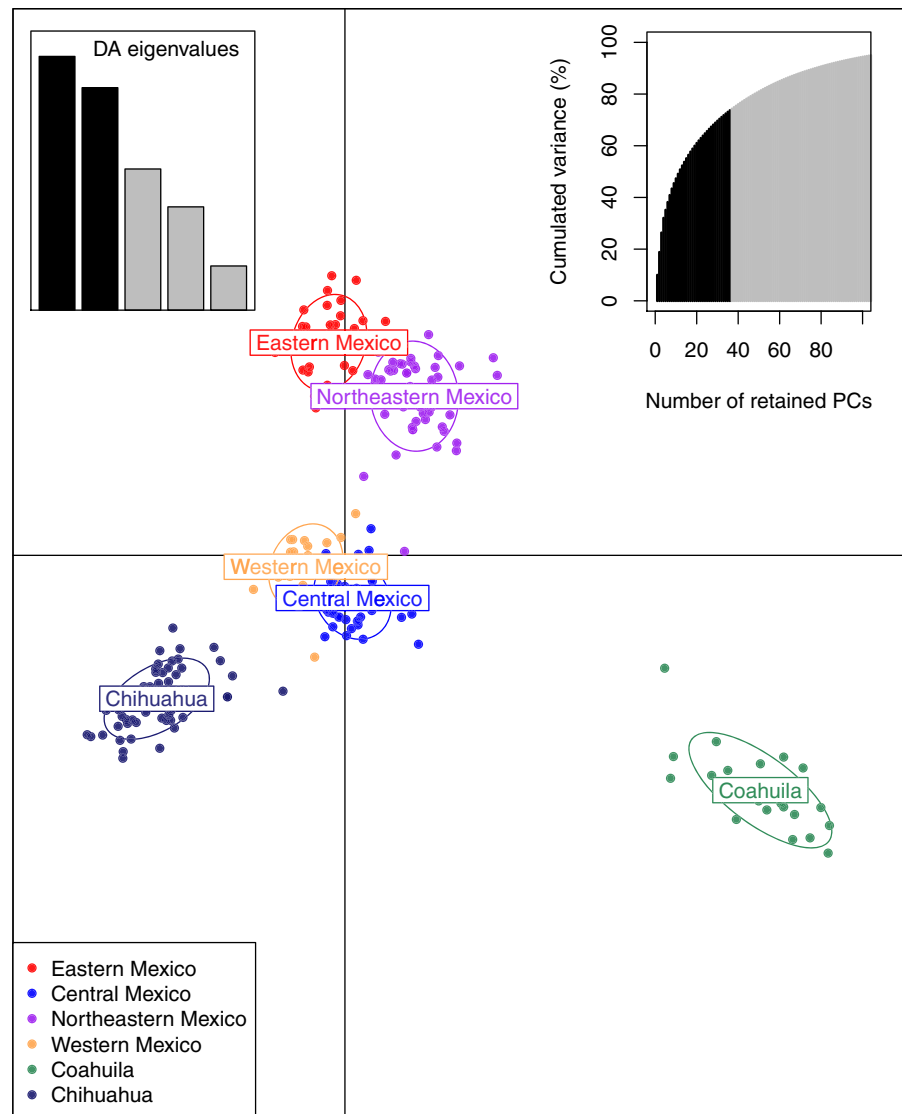
Genetic diversity within and among genetic groups

Supplementary Table 5 presents the allelic profiles of 247 unique samples. All markers were polymorphic. Table 3 displays the diversity parameters across all accessions for 15 SSR markers. A total of 338 alleles were detected across all markers with an average of 22.53 alleles across 247 accessions. The number of alleles ranged from 11 (VMC7f2) to 40 (VrZAG62). The number of effective alleles ranged from 4.04 (VVIn73) to 15.13 for VVMD27. Observed heterozygosity was lower than expected heterozygosity for all markers with a mean value of 0.61 and 0.86,

respectively (Table 3). Lower values of observed heterozygosity in conjunction with the results of the fixation index with most loci suggested high levels of inbreeding.

The SSR marker data was divided into six genetic groups previously assigned by the STRUCTURE program and allelic profiles were used to calculate statistical indices to determine the diversity within and among the six groups (Table 4). The number of alleles varied between different populations with a mean value of 7.77. The Coahuila group was the least diverse with an average of 4.2 alleles, and the Northeastern Mexico group was the most diverse with

Table 2 Summary of the key traits used to distinguish the geographic/genetic groups. The traits are based on the majority of the samples within that group

	Eastern Mexico	Western Mexico	Coahuila	Central Mexico	Northeastern Mexico	Chihuahua
Possible species	*V. cinerea* forms, *V. tiliifolia*	*V. bloodworthiana* and *V. cinerea* forms	*V. arizonica* forms, some *V. cinerea* traits, some *V. candicans* traits	*V. cinerea/ bloodworthiana* hybrids	*V. cinerea* forms	*V. arizonica* forms
Leaf shape	Cuneiform to heart-shaped	Cuneiform to heart-shaped	Cuneiform	Heart-shaped, few cuneiform, few three-lobed	Cuneiform to heart-shaped, wider than long	cuneiform
Key traits	Thick leaf, long apical tooth, dusky and olive-green color	Smooth surface, flat blade, red shoot tips, red back	Grey glaucous back, white shoot tips, tomentose, small clusters, wide petiolar sinus	Rugose, bullate, light to dark green	Rugose surface, thick, bullate, three lobed to entire	tomentose, thin paper-like blade, white shoot tips
Vein traits	Red to green veins	Green veins	Green veins, red petiole that bleeds into veins	Green veins	Red to green veins	green veins
Teeth	Short to no teeth	Short serrated teeth	Sharp short teeth	Short to no teeth	Short to no teeth	Short serrated teeth
Picture						

15.467 alleles (Table 4). The other four genetic groups (Eastern Mexico, Western Mexico, Central Mexico, and Chihuahua) had a comparable number of alleles that ranged from 7.53 to 8.86 (Table 4). The observed heterozygosity (H_O) for all groups was lower than the expected heterozygosity (H_E). Expected heterozygosity ranged from 0.47 to 0.79, with an overall estimate of 0.667. From all groups, the highest H_E was again the Northeastern Mexico group with a mean of 0.795 and the lowest H_E was Coahuila with 0.474. Fixation index ranged from 0.042 to 0.212, with the Eastern Mexico group being the lowest, and Western Mexico being the highest. A total of 127 private alleles were found across all markers within six groups. Private alleles for every group are shown underlined in Suppl. Table 6. The Northeastern Mexico group had by far the most private alleles (74 alleles) compared with the other groups. Overall, statistical indices found that the Northeastern Mexico group to be the most diverse and Coahuila the least diverse. The average value for

evenness was 0.698, with Central Mexico being the lowest with 0.649 and Chihuahua being the highest with 0.751.

Nei's genetic distance and F_{ST} were calculated to validate the results obtained from the different clustering analyses. Using the same STRUCTURE groups, pairwise comparisons of Nei's genetic distance and the F_{ST} were made. The pairwise comparisons for the six genetic groups are listed in Table 5. Nei's genetic distance had a wide range, from the lowest 0.483 for the pairwise estimate of Central and Western Mexico groups, and the highest of 1.879 for Northeastern Mexico and Coahuila. In general, Coahuila, Chihuahua, and Northeastern Mexico had the highest differentiation from the Eastern, Central and Western Mexico groups. Similarly, Coahuila had the highest pairwise F_{ST} values, showing the greatest genetic differentiation from all other groups (Table 5). The highest value was between Coahuila and Central Mexico (0.247), while the lowest pairwise estimate

Table 3 Summary of genetic diversity parameters across 247 unique accessions for each locus

Locus	No. of Alleles (Na)	Number of effective alleles (Ne)	Observed Heterozygosity (H_O)	Expected Heterozygosity (H_E)	Fixation Index (F)
VVMD31	31	13.85	0.72	0.93	0.22
VMC1b11	24	4.28	0.50	0.77	0.34
VVMD7	16	9.86	0.51	0.90	0.43
VVS02	20	6.60	0.68	0.85	0.20
VVMD28	21	10.89	0.67	0.91	0.26
VrZAG62	40	12.58	0.72	0.92	0.22
VrZAG79	20	11.02	0.70	0.91	0.23
VVMD27	32	15.13	0.77	0.93	0.17
VVIb23	19	6.28	0.67	0.84	0.20
VVMD21	20	7.70	0.54	0.87	0.38
VMC7f2	11	4.32	0.69	0.77	0.10
VVMD5	24	10.78	0.59	0.91	0.35
VVIn73	12	4.04	0.42	0.75	0.45
VVMD32	21	10.29	0.39	0.90	0.56
VVIp26	27	6.40	0.58	0.84	0.31
Mean	22.53	8.93	0.61	0.86	0.295

Na: No. of Alleles; Ne: Number of effective alleles; H_O: Observed Heterozygosity; H_E: Expected Heterozygosity; F: Fixation Index

Table 4 Summary of genetic diversity parameters in the six genetic groups

Pop		No. of Alleles (Na)	Shannon's Information Index (I)	Observed Heterozygosity (H_O)	Expected Heterozygosity (H_E)	Fixation Index (F)	Evenness
Eastern Mexico	Mean	7.86	1.42	0.60	0.64	0.04	0.68
	SE	0.91	0.14	0.05	0.04	0.05	0.02
Western Mexico	Mean	8.73	1.62	0.58	0.72	0.21	0.72
	SE	0.86	0.11	0.05	0.03	0.06	0.02
Coahuila	Mean	4.20	0.88	0.44	0.47	0.10	0.68
	SE	0.42	0.10	0.06	0.05	0.06	0.03
Central Mexico	Mean	8.86	1.48	0.58	0.66	0.11	0.64
	SE	1.01	0.13	0.04	0.04	0.02	0.02
Northeastern Mexico	Mean	15.46	2.07	0.67	0.79	0.15	0.68
	SE	1.26	0.13	0.04	0.03	0.02	0.03
Chihuahua	Mean	7.53	1.48	0.65	0.70	0.05	0.75
	SE	0.55	0.08	0.03	0.03	0.04	0.03
Total	Mean	8.77	1.49	0.58	0.66	0.11	0.69
	SE	0.50	0.06	0.02	0.01	0.02	0.01

was 0.077 for the Western and Central Mexico groups. Deviations from HWE were tested for every marker (Suppl. Table 7). Each population had markers that deviated significantly from the HWE. Populations that represented Northeastern, Chihuahua and Western Mexico had 12, 10 and 9 markers with deviation from HWE, respectively. The group from the Eastern Mexico, Coahuila, and Central Mexico had 2, 2 and

Table 5 Estimates of pairwise Nei's genetic distance (below the diagonal) and F_{ST} values (above the diagonal, bold) for the six genetic groups

	Eastern Mexico	Western Mexico	Coahuila	Central Mexico	Northeastern Mexico	Chihuahua
Eastern Mexico	–	**0.119**	**0.246**	**0.121**	**0.134**	**0.155**
Western Mexico	0.793	–	**0.208**	**0.077**	**0.100**	**0.111**
Coahuila	1.662	1.419	–	**0.247**	**0.205**	**0.231**
Central Mexico	0.696	0.483	1.715	–	**0.121**	**0.131**
Northeastern Mexico	1.389	1.143	1.879	1.272	–	**0.106**
Chihuahua	1.287	0.941	1.771	0.989	1.196	–

7 markers that deviated from equilibrium, respectively.

Discussion

This is the first report evaluating the population structure and genetic diversity of wild grape species with DNA-based markers in an important biogeographical region that contains 10 to 12% of all known species of plants and animals (Vanderplank et al. 2018). Mexico is a "mega-diverse" country where the Neartic and Neotropical regions overlap and form a transition zone. This transition zone contains five biogeographic provinces (Sierra Madre Occidental, Sierra Madre Oriental, Trans-Mexican Volcanic Belt, Sierra Madre del Sur and Chiapas) and houses about 31% conifer forests and 28% oak forest, while the remaining land is mostly cloud forests, riparian land, grasslands, and agricultural and urban land (Padilla et al. 2014). The region is under constant evolutionary pressure due to volcanic activity, climate change and most importantly, human activity (Mastretta-Yanes et al. 2015). Specifically, the Trans-Mexican Volcanic Belt has suffered substantial land use change and deforestation (García-Romero et al. 2012). Overexploitation of natural resources, introduction of invasive species, fires, pests and diseases are a few of the factors transforming 37 to 67% of Trans-Mexican Volcanic Belt forests (Padilla et al. 2014). The loss of native vegetation has been reported across the country where ranching and agriculture have expanded (Gómez-Aíza et al. 2017). In the last three decades, 16 out of 17 coastal states in Mexico lost about 5 million hectares of natural vegetation and the number of endangered species grew from 367 to 475

(Vanderplank et al. 2018). In this shifting environment, germplasm collections play a pivotal role in the conservation of flora for future generations. They also provide a window to the past capable of helping us understand historic and recent evolutionary changes.

Use of DNA markers for germplasm management

Accurate information is essential for efficient and cost-effective management of genetic resources. In this study, wild *Vitis* species from three germplasm collections in Mexico (Zumpahuacan, CICTAMEX, and UACh), the NCGR-Davis germplasm repository, and accessions collected from their native habitat across northern and central Mexico were evaluated and compared. Results from this study identified discrepancies in the accessions' passport data as well as duplicates of multiple accessions. Interestingly, duplicates of accession P-11 were found in different locations with different passport data (Supplementary Table 2). Based on the passport data, most of the duplicates of P-11 accession came from San Antonio de Cañada, Puebla, suggesting this may be the original location. This odd result may have been the result of P-11's human use. Wild grapevines have been used for different medicinal purposes in Central Mexico and have likely been through the selection process by local farmers and inhabitants (Franco-Mora et al. 2008b). It is possible that this specific accession had medicinal or culinary value to indigenous peoples and that it was distributed throughout Central Mexico. The other plausible explanation is that the large number of duplicated samples may very well be due to errors made during propagation and sampling. The fingerprint data of this study provides the foundation to identify duplicate accessions among collections and to

maintain only unique accessions to reduce management costs. These results also establish a DNA marker-based database of unique accessions that will facilitate future germplasm collection efforts.

Mexico harbors a wide diversity of wild *Vitis* species (Comeaux 1987, 1991) that comprise a valuable genetic resource for pest and disease resistances (Riaz et al. 2018b; Xu et al. 2008). These wild species are an important germplasm pool for the establishment of breeding programs for rootstock and scion development directed at combating climate change, drought and other biotic and abiotic stresses (Harvey et al. 2008). Thus far, only one species, *Vitis popenoei* J.L Fennell has been described as being in danger of extinction (Rzedowski and Calderón de Rzedowski 2005). However, given the changing land use in Mexico, the existence of many *Vitis* species may soon be threatened (Franco-Mora and Cruz-Castillo 2012), making it even more important to collect and preserve this unique germplasm.

Genetic differentiation and population structure

The results from this study provide important information that furthers the understanding of the genetic variation, hybridization patterns and overall population structure of Mexican *Vitis*. Clustering analyses with different programs identified six genetic groups within the study set that were distributed through different biogeographic regions (Figs. 1 and 2). The analysis showed hybridization patterns in specific locations. Most admix samples were observed within the Eastern Mexico, Chihuahua and Western Mexico genetic groups. Many of the admix accessions were from the NCGR-Davis (*b* and *DVIT* series), acquired from these regions in 1961 and the early 1990s. Eastern Mexico and Chihuahua also have a genetic continuum that extends to Northeastern Mexico and the Southwestern USA, respectively. It would be interesting to conduct a more extensive study and include a range of germplasm from the USA to gain better understanding of gene flow and speciation of the grape species.

The six genetic groups followed the pattern of physical barriers in the form of mountain ranges and deserts. The Northeastern group extended from the Texas bordering states of Nuevo Leon and Tamaulipas located in the Sierra Madre Oriental and had no physical barrier as it extended along the eastern coastline, to the Veracruzan province along the Gulf of Mexico. Results indicated that the Sierra Madre Oriental mountain range acted as a physical barrier to separate the Northeastern group from the western groups of Chihuahua and Coahuila (Fig. 2). Similarly, the Chihuahuan Desert is a region located between the Sierra Madre Oriental and Sierra Madre Occidental that extends into the United States. This region seems to also act as a physical barrier to keep the Chihuahua and Coahuila groups distinct and separate.

The Western and Eastern Mexico groups hybridization zones lay in the Trans-Mexican Volcanic Belt (Fig. 2) where the southern Sierra Madre Occidental and southern Sierra Madre Oriental end. It appears that the Trans-Mexican Volcanic Belt provides an east–west corridor for movement of germplasm. This trend has been observed in other plant and animal taxa studies (Escalante et al. 2007; Swenson and Howard 2005). Results from another study in the laboratory by Riaz et al. (2020) that analyzed genetic diversity of North American *Vitis* species also observed an east–west divide with the Sierra Madre Oriental as a phylogeographic barrier.

Pattern of genetic diversity within six groups

In general, we expected to see higher heterozygosity because wild grape species are dioecious (male or female flowered individuals) in nature and obligate out-crossers. However, we observed comparatively low heterozygosity. One possible explanation is wild grape species populations are fragmented due to deforestation and urbanization of their natural habitat that leads to more inbreeding. A similar trend has been seen in *V. sylvestris* wild populations in many parts of the world (Bacilieri et al. 2013; de Andrés et al. 2012; Riaz et al. 2018a). In contrast, most of the measured parameters indicated that the Coahuila group had the lowest values for all diversity indices. This relatively low genetic diversity might be due to a small sample size collected from a narrow region in comparison to the larger region that the other groups cover. The Coahuila group was also the only group that showed pairwise F_{ST} values above 0.20 indicating that had less outbreeding with other populations. This distinction may be due to the geographic isolation resulting from the Sierra Madre Oriental which separates the Coahuila group from the Northeastern region. Additionally, viticulture has been practiced in Coahuila

since 1597 (Muñoz Rodríguez 1986). The deforestation and establishment of agricultural land creates man-made barriers that could also have contributed to the isolation of this wild grapevine population (Riaz et al. 2018a).

The diversity parameters like observed and expected heterozygosity detected high levels of polymorphism within the Northeastern Mexico group, which also had the highest number of private alleles. This group also had the maximum number of markers that deviated from the HWE indicating that it was more impacted by evolutionary forces such as migration, genetic drift, selection and mutation. These results were expected as different clustering analyses revealed a wider geographic range for this group extending to the Gulf of Mexico and admixing with accessions from the eastern and central Mexico (Fig. 2).

Genetic distance analyses such as PCoA, DPAC, and Nei's values were valuable tools to observe genetic differences between the groups. The Nei's genetic distance measures showed which groups had a lower or higher genetic differentiation (Hartl et al. 1997; Wright 1965). For example, Central, Eastern and Western Mexico groups had smaller estimates (Table 5), which was also observed in the PCoA that found these groups are in the same quadrant (Fig. 4) and are closely related in the dendrogram (Fig. 3). Similarly, the relatively high F_{ST} values indicated genetic differentiation among groups that cannot be fully observed in the PCoA, but it is very clear in the DAPC (Fig. 5). For example, Coahuila had the highest pairwise F_{ST} values to all groups, which was visible in the DAPC which positioned Coahuila in a separate quadrant. It can be hypothesized that the DAPC may show a geographic continuity between Eastern and Northeastern Mexico groups and between Central and Western Mexico groups. Displaying the DAPC helped to visualize the differences among groups (Jombart et al. 2010) as the data was analyzed using the predefined genetic groups, contrary to just observing the differences between all accessions in the PCoA which are not sorted into groups before the analysis.

Morphologic characterization and presence of Vitis species in Mexico

The study of North American Vitis spp. has been discordant leading to two trains of thought: The first focused on defining species, and the second on consolidating them in subgroups within fewer distinct species (Moore 1991). The presence of species in Mexico like V. arizonica., V. blancoii Munson, V. tiliifolia, Vitis jaegeriana Comeaux, V. cinerea and V. berlandieri, among others, have been established (Bailey 1934; Comeaux 1991; Comeaux and Lu 2000; Moore and Giannasi 1994; Munson 1909; Rzedowski and Calderón de Rzedowski 2005). However, there are differing opinions as to the number of Vitis species, and their range in Mexico with the caveat that some species have been misidentified. The absence of comprehensive morphological and phylogenetic information, and hybridization where species ranges overlap, also complicates the identification of a given species. Additionally, there is no consensus as to what classifies as a new species, a hybrid or a different "form" or "variety" of a specific species. Although in this study, we attempted to use morphological characteristics to define species, it is duly noted that identification by morphology is challenging. It was observed species assignment did not match perfectly with the identified STRUCTURE genetic groups. Species assignment was used as a tool to the genetic characterization, and was not considered as a definitive result and did not align perfectly with the obtained genotype. However, morphology played a role in the analysis and supported with identification of hybridization zones.

The collection notes identified cordate leaf form as the most common leaf feature (Table 2). Cordate leaves with diminutive teeth and a rugose leaf surface are typical features of V. cinerea. Based on the morphological features of accessions in this study, it can be inferred that V. cinerea and its hybrids are the most widely spread species in Central Mexico from east to west, Puebla and Veracruz, and beyond to Northeastern Mexico (Table 2). The presence of this species in this geographic range has been previously described (Franco-Mora et al. 2012; Moore 1991). Most of the accessions in the Zumpahuacan and CICTAMEX germplasm collection were obtained from this region, and thus have a strong representation of this species. The Puebla region has a humid temperate climate with rainfall year-round. Vitis cinerea Engelm.ex Millardet is usually found in moist habitats, river banks and pond margins (Bailey 1934). Other studies that evaluated the V. cinerea germplasm and included Mexican accessions also identified them

to be unique and genetically distinct from the *V. cinerea* found in Texas and other gulf coastal states (Heinitz et al. 2020; Riaz et al. 2020). From the results of this study, it can also be deduced that *Vitis cinerea* has been moving through the northern Sierra Madre Oriental using it as a corridor for a long time. Nonetheless, it is still inconclusive whether *V. cinerea* moved in a specific direction or if it was bidirectional. In Mexico it appears it hybridized with other native species in the region moving to the Trans-Mexican Volcanic Belt where it could hybridize with *V. bloodworthiana* to the western side, and with *V. tiliifolia* to the east. Extensive hybridization of *V. cinerea* over centuries has resulted in different forms in different regions. This scenario is similar to the case of *V. berlandieri*, which is now considered to be a variety of *V. cinerea* (*V. cinerea* var. helleri) found on the limestone soils of central Texas (Walker et al. 2019).

Vitis arizonica was identified in the southwestern US and northern Mexico in the 1870s. Previous studies have identified this species in Chihuahua, Coahuila, Durango and Sonora (Bailey 1934). This species was described as a grayish shrubby vine that does not climb high, ashy grey young shoots, broad cordate-ovate leaves with a broad U-shaped petiolar sinus with small sharp uneven teeth and mostly found in canyons where water is available (Bailey 1934). From the described morphology, the Chihuahua and Coahuila accessions appear to be either pure *V. arizonica* or hybrids between it and other species. Coahuila's accessions appear to have some *V. cinerea* and *V. candicans* traits that might be indicative of hybridization. Northeastern Mexico and Coahuila are contiguous regions that could allow intergradation between species. However, accessions in the Northeastern group look more similar to the accessions located in Central Mexico, and Coahuila looks more similar to accessions from Chihuahua. Although Northeastern Mexico and Coahuila are relatively close, they are separated by the Sierra Madre Oriental mountain range, which seems to be a strong physical barrier to gene flow. In contrast, Central Mexico and Northeastern Mexico are geographically distant, but there is no physical barrier. The Sierra Madre Oriental and the Trans-Mexican Volcanic Belt have acted as a conduit for gene flow from these regions.

Vitis tiliifolia is reported to be the most widely spread species in Central Mexico (Guerrero, Hidalgo, Jalisco, Michoacán, Morelos, Nayarit, San Luis Potosí, Puebla and Mexico State) and Southern Mexico (Campeche, Chiapas, Oaxaca and Veracruz). This species has been documented south to Colombia and the Antillas (Rzedowski and Calderón de Rzedowski 2005). *V. tiliifolia*, also known as *V. caribeae*, grows in tropical low and highlands, in acidic soils with high levels of organic matter (Galindo-Tovar et al. 2019). It has been described as having bifurcated tendrils, long apical tooth, very hairy, serrated leaves, sometimes lobed, and light green on the back of the leaf. *Vitis blancoii* Munson and *Vitis biformis* Rose are other reported forms of *V. tiliifolia* (Rzedowski and Calderón de Rzedowski 2005). Multiple studies have described its presence in Puebla and Veracruz (Comeaux 1987; Galindo-Tovar et al. 2019). Although, most of the accessions in this study were collected from Tamaulipas, Veracruz and Puebla in the Sierra Madre Oriental, morphological features of *Vitis tiliifolia* were also identified in the Eastern, Northeastern and Central Mexico groups, groups with leaf features of *V. cinerea*. From these results, we can infer that the ranges of *V. tiliifolia* and *V. cinerea* overlap allowing hybridization. However, more sampling from southern Mexico is necessary to identify *V. tiliifolia*'s entire range and the extent of hybridization with *V. cinerea*.

Previous studies have reported that *V. bloodworthiana* could only be found at high elevations in the Sierra Madre Occidental of Sinaloa and Durango with non-sympatric range with other *Vitis* species (Comeaux and Lu 2000). Comeaux (1991) described *V. bloodworthiana* as morphologically similar to *V. arizonica*. This species has flat long-cordiform leaves, usually 3-lobed, with serrate to crenate margins and glabrous on both sides (Comeaux 1991). In this study, we identified that not only were accessions from Durango and Nayarit similar to *V. bloodworthiana*, but many accessions from Central Mexico also possess *V. bloodworthiana* characteristics (Table 2 and Suppl. Table 4). From these results, we can infer that the overlapping region of the Trans-Mexican Volcanic Belt and the southern part of the Sierra Madre Occidental is a potential hybridization zone.

Based on the morphological characteristics of the collected samples, none of the samples had typical *V. berlandieri* or *V. riparia* leaf morphology. Preliminary results from another study evaluating a large collection of North American species also did not find

association of *V. berlandieri* accessions to the germplasm collected in Mexico (Heinitz et al. 2020). The absence of these species in Mexico might be due changes in land use and the diversion of water from southwestern US rivers and streams that once flowed to Northern Mexico (Caciano et al. 2012). Other studies have also expressed concerns as to whether these species were ever present in Mexico (Rzedowski and Calderón de Rzedowski 2005).

Conclusion

This is the first assessment of genetic diversity of Mexican wild grape species based on SSR marker fingerprint data and provides a framework to manage unique germplasm in different collection sites in Mexico. Population structure and genetic diversity analyses identified six genetic groups that are separated by mountain ranges of the Sierra Madre Oriental with an east–west divide and the Trans-Mexican Volcanic Belt act as a corridor for gene flow among different grape species. *Vitis cinerea* was the most prevalent species with a large geographical range. It hybridizes with other native species like *V. bloodworthiana* to the western side, and with *V. tiliifolia* to the east, possibly forming other types that are difficult to identify solely by their morphological features. Clustering analyses and diversity indices indicated that the Coahuila and Chihuahua genetic groups were more isolated and morphological features revealed them to be pure forms of *V. arizonica* and its hybrids. Additional grape germplasm from different parts of Mexico is needed to identify the extent and spread of different grape species, and to identify hybrid zones and their role in grape speciation. This study attempts to highlight the importance of *Vitis* conservation not only for grapevine breeding, but ecosystem services wild grapevines serve to the Mexican indigenous communities.

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