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Technical note

Genetic analysis of Oaxacan Mixteco Creole cattle

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Abstract:

The Mixteco Creole cattle is a little explored genetic resource, which, however, has great value due to its potential to be used in production systems that are respectful of the environment and adaptable to its conditions. The identification and characterization of this local resource is essential for its conservation and improvement. For this reason, in the present study it was carried out the analysis of the diversity and genetic relationships of the Mixteco Creole cattle population of Oaxaca, using 19 microsatellite DNA markers and 32 reference cattle populations belonging to the BIOBOVIS consortium of the CONBIAND Network. The mean number of alleles detected was 8.8 ± 2.1 and the estimated effective number of alleles was 4.5 ± 1.2. The genetic diversity represented by the expected (0.7700 ± 0.0682) and observed (0.7170 ± 0.0998) heterozygosity values was within the range of

estimators obtained in previous studies with local cattle populations using microsatellite markers. An analysis of the population structure revealed a predominant influence of Iberian germplasm (*Bos taurus*). There is also a close relationship between the Mixteco Creole and the rest of the Mexican Creole cattle populations, with the exception of the Tropical Dairy Creole.

Keywords: Conservation, Creole cattle, Genetic characterization, Microsatellites.

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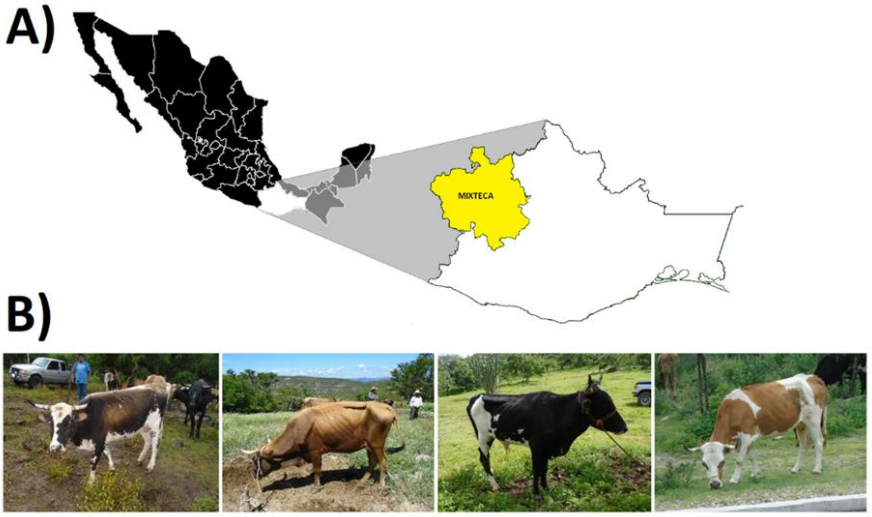
Creole cattle represent a genetic resource of great importance for the supply of food and raw materials in areas with extreme climatic conditions, scarce food resources and high incidence of infectious and parasitic diseases[(1)], and potentially contribute to hunger and poverty reduction, as well as to sustainable development[(2)]. However, the inability to appreciate the real biological, economic and cultural value of these animals has caused an aggressive extension of highly selected breeds, causing a constant erosion that endangers the existence of these resources and, thereby, an irreparable loss of genetic variability, which could be of great value to face the effects of climate change.

In the state of Oaxaca, there is a population of Creole cattle located in the Mixteca region (Figure 1A), known as Mixteco Creole. Phenotypically, they are medium-sized animals, with an average height at the withers of 1.03 ± 0.16 m and an average weight of 176 ± 51.48 kg (parameters reported for an age of 1 to 3 yr)[(3)]; their coat can be uniform black or red, or black- or red-spotted (Figure 1B). The origin of the Mixteco Creole cattle dates back to colonial times, having probably been brought by the first Spaniards for the construction of the convents along the Oaxacan territory, on what is currently known as the Dominican Route[(4)].

Figure 1: A) Geographic location of the Mixteca region of Oaxaca. **B)** Mixteco Creole bovine cattle



The image shows the coloration and morphostructural characteristics of the Mixteco Creole bovine cattle

Since its introduction into the region, the Mixteco Creole cattle have successfully adapted to the geographical and environmental conditions prevailing in the area, which is characterized by a complicated orography and fluctuations in the availability of food and water. However, these cattle are capable of being productive under such conditions, which makes them suitable for developing environmentally friendly, resilient production systems that are able to cope with changes in the environment, especially with the current trend in Latin America towards the development of more intensive and sustainable production systems[5].

According to the Food and Agriculture Organization of the United Nations (FAO), the identification and genetic characterization of local livestock is an essential step in the conservation and use of these genetic resources[6]. In this regard, microsatellite molecular markers have proven to be a highly effective tool to genetically characterize Creole cattle populations in the Americas[7–10], Therefore, this study evaluated the genetic diversity of the Mixteco Creole cattle population and their genetic relationships with other local and specialized cattle populations, using 19 microsatellite markers and 32 reference populations in order to generate information on the conservation status of this valuable local genetic resource.

A total of 40 adult Mixteco Creole cattle (29 females and 11 males) were selected and identified based on phenotypic characteristics of Creole cattle previously reported in the literature, including color pattern, size, and zoometric parameters[3,11]; those individuals that exhibited phenotypic characteristics typical of Zebu breeds were discarded. In order to avoid close kinship relationships among the selected individuals, only one specimen was included for each one of the cattle production units sampled, geographically separated in different

communities of the Oaxacan Mixteca (17°48′00″ N, 97°46′00″ W), in addition to confirming, through an interview, the absence of genetic connections (use of stallions) between the production units. The sample size was defined taking as a reference the information published for population genetic studies using microsatellite molecular markers[12], as well as the sample size suggested by FAO for genetic characterization studies of local livestock populations using microsatellites (n=25 to 40)[13].

The biological material consisted of whole blood samples with anticoagulant (EDTA) obtained by aseptic puncture of the jugular vein, from which nucleic acids were extracted using the ReliaPrep™ Blood gDNA Miniprep System kit (Promega), following the manufacturer's instructions. A panel of 19 microsatellite markers recommended by FAO-ISAG was used for the genetic analysis of the population[14] (Table 1), having been amplified by PCR and processed in an ABI377XL capillary sequencer (Applied Biosystems), with subsequent allelic typing, following the methodology established at the Laboratory for the Improvement and Conservation of Animal Genetic Resources of the University of Cordoba, Spain[7].

Table 1: Allele frequencies observed in the Mixteco Creole Cattle population

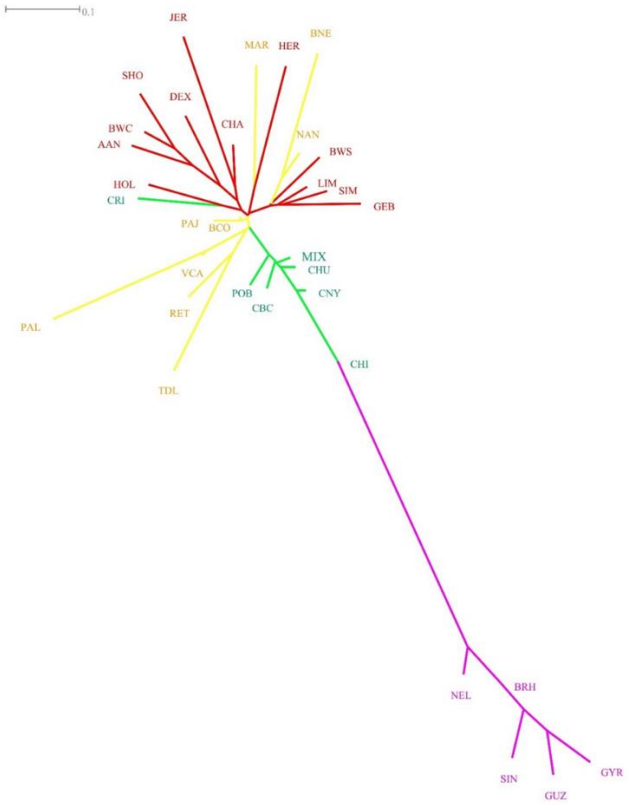
BM1818		**BM1824**		**BM2113**		**CSRM60**		**CSSM66**	
Allele	*Frec.*	*Allele*	*Frec.*	*Allele*	*Frec.*	*Allele*	*Frec.*	*Allele*	*Frec.*
260	0.0132	179	0.2750	126	0.0385	91	0.0125	179	0.0132
262	0.0395	181	0.1750	128	0.0513	93	0.1500	181	0.0921
264	0.3553	183	0.3875	130	0.0769	95	0.0125	183	0.1974
266	0.1842	185	0.0125	132	0.0256	97	0.1375	185	0.0789
268	0.3421	189	0.1500	134	0.0769	99	0.0250	187	0.0132
270	0.0526			136	0.2436	101	0.0125	189	0.2895
272	0.0132			138	0.3077	103	0.4125	191	0.0132
				140	0.1026	105	0.1875	193	0.1842
				142	0.0769	111	0.0375	197	0.1184
						113	0.0125		
ETH003		**ETH010**		**ETH185**		**ETH225**		**HAUT27**	
Allele	*Frec.*	*Allele*	*Frec.*	*Allele*	*Frec.*	*Allele*	*Frec.*	*Allele*	*Frec.*
103	0.0263	209	0.1667	220	0.0286	139	0.2000	128	0.0135
109	0.1053	211	0.0128	222	0.0571	141	0.0125	140	0.0270
115	0.0658	213	0.1026	226	0.0571	143	0.1125	142	0.0811
117	0.3421	215	0.1154	228	0.6000	145	0.0250	146	0.0135
119	0.1974	217	0.3333	230	0.0143	147	0.2625	148	0.5405
123	0.0658	219	0.1923	232	0.1000	149	0.2875	150	0.2297
125	0.1711	221	0.0256	234	0.1143	151	0.0125	152	0.0676
129	0.0263	223	0.0513	236	0.0143	153	0.0250	154	0.0270
				240	0.0143	157	0.0625		
HEL009		**ILSTS006**		**INRA32**		**INRA63**		**MM12**	
Allele	*Frec.*	*Allele*	*Frec.*	*Allele*	*Frec.*	*Allele*	*Frec.*	*Allele*	*Frec.*
149	0.0128	287	0.0556	168	0.0152	175	0.4250	105	0.0132
151	0.0128	289	0.0417	174	0.0152	177	0.3250	109	0.0132
153	0.3077	291	0.2361	176	0.1364	179	0.0125	117	0.1579
155	0.0641	293	0.2778	178	0.2879	183	0.2000	119	0.1184
157	0.0256	295	0.1111	180	0.3485	185	0.0375	121	0.2368
159	0.0256	297	0.2639	182	0.0303			123	0.1184
161	0.2308	299	0.0139	184	0.1212			125	0.0526
163	0.1154			186	0.0152			129	0.0132
165	0.0513			188	0.0303			131	0.0263
167	0.0513							133	0.1974
169	0.0128							135	0.0395
171	0.0897							139	0.0132

SPS115		**TGLA053**		**TGLA122**		**TGLA227**	
Allele	*Frec.*	*Allele*	*Frec.*	*Allele*	*Frec.*	*Allele*	*Frec.*
242	0.0375	151	0.0658	134	0.0250	79	0.0769
244	0.4875	153	0.0395	140	0.0125	81	0.0256
246	0.0625	157	0.2105	142	0.0375	83	0.1282
248	0.1875	159	0.1711	144	0.0375	85	0.3077
250	0.0250	163	0.0526	146	0.0750	87	0.0128
252	0.0875	165	0.0921	148	0.0125	89	0.0641
254	0.0250	167	0.1842	150	0.3875	91	0.0641
256	0.0875	169	0.1447	152	0.2875	93	0.0128
		175	0.0263	154	0.0125	95	0.0385
		181	0.0132	156	0.0125	99	0.2692
				160	0.0625		
				168	0.0125		
				174	0.0250		

The information generated from allelic typing was used to calculate allele and genotypic frequencies for each microsatellite marker using the MSTools add-in for Excel (Genetics Dept, TCD, Ireland). The number of alleles per locus, effective number of alleles, observed and expected heterozygosity, polymorphic information content (PIC), and F_{IS} inbreeding coefficient were estimated with Popgene v 1.32[15]. The Hardy-Weinberg equilibrium test was carried out with Arlequin v 3.1[16].

The analysis of the population structure and genetic relationships was carried out using allelic information from 32 reference populations previously reported in the literature (Figure 2)[17], belonging to the BIOBOVIS Consortium (https://BIOBOVIS.jimdofree.com/) of the Network for the Conservation of Local Domestic Animal Biodiversity (CONBIAND). The reference populations were divided into four groups according to their origin or specialization (Mexican Creoles, Iberians, specialized Europeans, and Zebu). The genetic distance between pairs of NEI populations (DST) was estimated with the Arlequin v3.1 software. Based on the information generated in the genetic distance matrix, a graphical representation in the form of a phylogenetic tree was created using the SplitsTree v.4.14.16 software, with the Neighbor-Joining method. Finally, the Structure software was utilized[18] to infer the population structure, using the following parameters: 100,000 warm-up iterations followed by 1'000,000 Markov chain-based Monte Carlo (MCMC) iterations. A total of 32 different runs (K2 to K33) were performed to estimate the most probable number of existing clusters. The optimal K value was estimated by the modal value method of the Delta K distribution, using the formula Delta K = mean (|L"(K)|) / sd (L(K)).

Figure 2: NEI's genetic distance (unbiased distance) tree using the Neighbor-Joining method



The circle shows the location of the Creole populations within the genetic distance tree (except for the Tropical Dairy Creole), revealing their intermediate position between the European *Bos taurus* breeds and the *Bos indicus* breeds. Populations: MIX (Mixteco Creole Bovine), CRI (Tropical Dairy Creole), POB (Poblano Creole), CBC (Baja California Creole), CHU (Chihuahua Creole), CNY (Nayarit Creole), CHI (Chiapas Creole), TDL (Fighting), RET (Very dark), BCO (Red-spotted), BNE (Black-spotted), MAR (Marshland), PAJ (Straw-colored), NAN (Andalusian Black), VCA (Canary Island cattle), PAL (Palmera), AAN (Aberdeen Angus), BWC (British White Cattle), HER (Hereford), JER (Jersey), SHO (Shorthorn), DEX (Dexter), BWS (Brown Swiss), CHA (Charolais), HOL (Holstein- Fresian), LIM (Limousin), SIM (Simmental), GEB (Gelbvieh), GYR (Gyr), BRH (Brahman), SIN (Sindi), GUZ (Guzerat), NEL (Nelore).

Table 1 shows the results of the calculation of allele frequencies for each of the 19 *loci* analyzed in the Mixteco Creole cattle population. Polymorphic variation was observed in all the *loci* analyzed. In total, 168 alleles were detected distributed among the 19 microsatellites, representing a mean number of alleles of 8.8 ± 2.1, with TGLA122 (Na= 13), MM12 (Na= 12) and HEL009 (Na= 12) markers having the highest number of alleles (Table 2). With regard to the effective number of alleles, a mean of 4.5 ± 1.2 alleles was observed, while the average polymorphic information content was 0.7286 ± 0.0748. The observed heterozygosity value was 0.7170 ± 0.0998, and the expected heterozygosity was 0.7700 ± 0.0682. Table 2 shows the results of the main genetic diversity parameters for each of the microsatellite

markers evaluated. The HAUT27, ILSTS006, and TGLA227 markers were the only *loci* in Hardy Weinberg disequilibrium ($P<0.05$). On the other hand, 15 of the 19 microsatellite markers showed positive F_{IS} values, and the remaining 4 showed negative values; however, most of them were separated from the zero value, obtaining a mean F_{IS} value of 0.058, with TGLA227 and BM1818 markers showing the greatest deviation from the positive F_{IS} value and CSRM60 marker showing the greatest deviation from the negative F_{IS} value.

Table 2: Genetic diversity parameters

Marker	Na	Ne	PIC	Ho	He	F_{IS}	*P* value
BM1818	7	3.5479	0.6695	0.5789	0.7277	0.1938	0.0640
BM1824	5	3.5834	0.6728	0.6500	0.7301	0.0984	0.7939
BM2113	9	5.3462	0.7907	0.7949	0.8235	0.0222	0.8494
CSRM60	10	4.0100	0.7195	0.8250	0.7601	-0.0991	0.6078
CSSM66	9	5.3780	0.7895	0.8684	0.8249	-0.0668	0.4087
ETH003	8	4.8456	0.7673	0.7632	0.8042	0.0384	0.8776
ETH010	8	4.9223	0.7704	0.7436	0.8072	0.0668	0.1559
ETH185	9	2.5574	0.5860	0.5143	0.6178	0.1555	0.2228
ETH225	9	4.7690	0.7597	0.7750	0.8003	0.0194	0.9550
*HAUT27**	8	2.7939	0.6022	0.6216	0.6509	0.0319	0.0042
HEL009	12	5.5410	0.7990	0.8205	0.8302	-0.0012	0.8609
*ILSTS006**	7	4.5474	0.7458	0.8056	0.7911	-0.0326	0.0136
INRA32	9	4.1644	0.7244	0.6667	0.7716	0.1227	0.4309
INRA63	5	3.0505	0.6101	0.6000	0.6807	0.1074	0.4384
MM12	12	6.5045	0.8283	0.8421	0.8575	0.0049	0.2746
SPS115	8	3.3934	0.6763	0.6750	0.7142	0.0430	0.7280
TGLA053	10	6.8274	0.8366	0.7368	0.8649	0.1367	0.1248
TGLA122	13	4.0455	0.7211	0.7000	0.7623	0.0702	0.5162
*TGLA227**	10	4.9951	0.7743	0.6410	0.8102	0.1985	0.0017

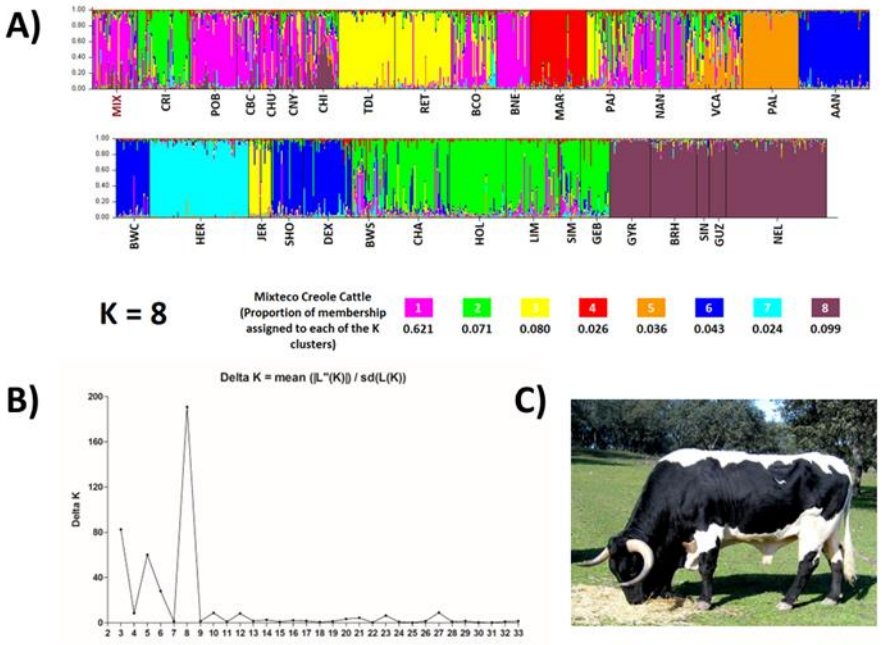
Na= number of alleles; Ne= effective number of alleles; PIC= polymorphic information content; Ho= observed heterozygosity; He= expected heterozygosity; F_{IS}= coefficient of inbreeding; *P* value = Hardy Weinberg equilibrium significance.

The genetic distance analysis revealed that the Mixteco Creole cattle clusters with Mexican Creole populations (Figure 2), showing a smaller distance with regard to Creole cattle from

Chihuahua (D= 0.046), Baja California (D= 0.064), and Puebla (D= 0.078). As for the rest of the population groups, the Mixteco Creole exhibited a smaller genetic distance in relation to the Red-spotted population (D= 0.103), belonging to the Spanish landrace group, as well as with respect to the Limousin breed (D= 0.190), which is one of the specialized European breeds. The greatest genetic distance was observed with each of the *Bos indicus* breeds (Nelore 0.588 – Gyr 0.734).

Finally, the results of the analysis of the population structure through the assignment test with Structure software (Figure 3A) and the subsequent calculation of the optimal K value using the modal value method of the Delta K distribution (Figure 3B), revealed that the optimal K was 8. The proportions of assignment to each cluster for Mixteco Creole are shown in Figure 3A. According to the results of K8, it is observed that, with the exception of the Dairy Creole population, the rest of the Mexican Creole populations, including the Mixteco Creole, exhibit a higher percentage of assignment to cluster 1, which includes the local Black-spotted, Red-spotted, Andalusian Black, and Straw-colored Spanish populations (Figure 3). The rest of the Mixteco Creole cattle genome was distributed as follows: 14.2 % with the rest of Spanish landraces, 13.8 % with specialized European breeds, and 9.9 % with the cluster of Zebu populations.

Figure 3: A) Results of the Bayesian Structure analysis for the Mixteco Creole Cattle population, using 32 reference populations and a total of 8 inferred clusters (K8). **B)** Estimation of the optimal K using the modal value method of the Delta K distribution. **C)** Individual of the Black-spotted breed



(Source of the photograph: National group of Black-spotted and Red-spotted cattle breeders' associations).

Local populations play a key role in livestock breeding, as they have been the basis for the development of specialized breeds and today constitute a reservoir of genetic diversity that must be preserved[19]. In this sense, the Mixteco Creole cattle represents a local livestock resource of great value that must be preserved, for which purpose its genetic evaluation is essential.

The results of allelic diversity, represented by the mean number of alleles observed, showed a similarity with the data reported in previous studies in bovine populations, when compared with the mean values per population [6.92 ± 0.99][7], [6.78 ± 1.88][8], [8.31 ± 2.10][20], however, they are lower when compared with the mean number of alleles detected considering the Creole populations as a group [14.21 ± 3.74][8], [15.5 ± 0.9][17]. The difference observed in the mean number of alleles when compared at the group level is due to the heterogeneity of Creole populations in the Americas, which has been confirmed in several studies using autosomal and mitochondrial polymorphisms[21-23]. This heterogeneity is probably the result of several factors such as differences in the origin of the populations[17], differentiation by geographical location[18], as well as the process of genetic drift and the contribution of animals of different origins, which have been mixed at some point with the Creole populations, as has been described with the introgression of Zebu, African and British breeds[21,23-24]. This data should be taken with caution, since the heterogeneity observed could also reflect the state of threat to the population, due to dilution as a result of intensive interbreeding or as a consequence of isolation and abandonment[17].

The values of observed and expected heterozygosity represent a measure of genetic diversity in a population; however, they are estimated using different data. On the one hand, expected heterozygosity is estimated based on allele frequencies, while observed heterozygosity is estimated from genotypic frequencies, so the differences observed between both estimates can be an indicator of the level of inbreeding in the population[25]. The present study showed a difference between heterozygosity values; the observed heterozygosity was lower than the expected heterozygosity, suggesting a tendency towards inbreeding, as could be subsequently verified in the estimation of the F_{IS} value. On the other hand, when comparing the expected heterozygosity values and the effective number of alleles with the values reported for the Creole breeds using a similar panel of microsatellites[10,17,20], a correspondence in the results was observed. The effective number of alleles represents the number of alleles expected in a population with the same heterozygosity but with equally distributed allele frequencies[26]; therefore, if the allele frequencies are highly unbalanced and only some alleles are in the majority, the effective number of alleles will tend to be lower[27], as was observed in the Mixtec Creole population, among which certain alleles are more frequent. The relevance of estimating the number of alleles and effective number of alleles lies in the fact that these data can be used as a conservation criterion, since allelic diversity can have important implications in the response to selection for adaptation to changing environments[28], This is of great importance when talking about Creole populations, as they are considered reservoirs of

genetic information to face potential environmental alterations due to climate change, so it is of great importance to implement measures for the conservation of allelic diversity.

The polymorphic information content exceeded 0.5 for all markers, which, according to the scale proposed by Botstein[29], indicates that the microsatellites evaluated are highly informative, and could be used for further genetic diversity monitoring studies or parentage testing. Regarding Wright's fixation index, the estimation of this parameter provides a measure of the degree of inbreeding of individuals with respect to the population to which they belong[30]. Although generally positive, the F_{IS} value can be negative if inbreeding is systematically avoided within populations[31]. In the present study, the estimated F_{IS} values were mostly positive, far from zero, suggesting a tendency for heterozygote deficiency inbreeding. The result observed in the population, in which the deviation of F_{IS} with respect to the zero value is positive can be attributed to the condition of the domestic population, where mating is not random and the proportion of males is lower compared to females, in addition to being long production systems with few animals and whose replacements are usually obtained within the same production units, which predisposes to inbreeding[32-33]. On the other hand, with respect to the Hardy-Weinberg equilibrium test, of the 19 markers analyzed, only markers HAUT27, ILSTS006 and TGLA227 were found to be out of equilibrium; this data increases the reliability of the results obtained in the study and also suggests that the population is not being subjected to perturbing forces that cause significant changes in their genotypic frequencies[34].

Genetic relationships between populations were analyzed using genotypic information from 32 breeds belonging to the BIOBOVIS consortium of the CONBIAND Network, which were selected for their potential relationship with Mixteco Creole cattle. We used 6 Mexican Creole populations, 9 Spanish local populations, which could be found among the founding populations of Latin American cattle, 12 European specialized breeds, and 5 Zebu breeds. As Figure 2 shows, the results of the genetic distance calculation show that the Mixteco Creole groups with the Mexican Creole populations of Chihuahua, Nayarit, Baja California and Puebla, separated from the European and Zebu cattle groups. This distribution has been reported in previous work with Latin American Creole cattle[17,35] This shows that, like other Creole populations in the Americas, the Mixteco Creole has an identity closely related to other Creole cattle due to their shared origin; this identity has been preserved despite the geographic distance between the populations. Interestingly, the smallest genetic distance to a non-Creole population was observed with the Red-spotted population, which has been described as one of the possible founding populations of American Creole cattle[20]. Currently, in the Mixtec region, the Zebu breeds have been replaced by *Bos taurus* cattle of better temperament, as, according to producers, crossbreeding between Zebu and Creole cattle generated individuals with a temperament that was difficult to manage, while European cattle, having been subjected to more intensive selection for docility and ease of management[36], do not exhibit this issue. This fact has probably prevented the introgression

of Zebu germplasm into the Creole population from increasing, maintaining the genetic distance between these populations.

Regarding the population structure, Bayesian analysis was carried out with Structure software, calculating different values of K (2 - 33), with the subsequent estimation of the optimal K (K= 8). A model with K= 8 populations was suggested, because it was associated with a higher probability (Figure 3B), suggesting that there are closely related races or groups (Figure 3B)[7]. For a K = 8, the results indicate that Mixteco Creole cattle maintain a relatively uniform population structure, sharing 62 % of their genome with Mexican Creole populations, except for the Tropical Dairy Cattle. Similarly to what was reported for Ecuadorian local breeds, a contribution of Red-spotted and Straw-colored cattle and a low relevance of Marshland cattle were observed[20], which suggests that the Mixteco Creole is integrated in its origin with Latin American Creole populations and reinforces the theory that these breeds are part of the founding populations of Creole cattle in Latin America, since, as has been described in other works, most of the Spanish cattle that gave rise to the Creole populations in America came from southern Spain[20]. In addition, this cluster also includes the Spanish Black-spotted (Figure 3C) and Andalusian Back landraces, which is interesting because the characteristic coloration patterns of the Mixteco Creole are similar to the coloration patterns of these Iberian populations, mainly in the Black-spotted population, whose genome percentage assigned to this cluster was 87.4 %. The Mixteco Creole genome composition for K= 8 exhibited genetic heterogeneity, which has been previously described in other Creole populations using mitochondrial, autosomal and Y chromosome markers[22,24,37]. The results confirm that the influence of Iberian cattle is predominant in the Mixteco Creole cattle, as is the case in the Creole populations of the Americas, which retain genetic signatures of their Iberian ancestry[21]. However, it is also possible to infer that there may have been recent contributions of exotic germplasm belonging to cattle of different origins, which, based on the percentages of allocation observed in lower proportions, would be European and Zebu. The presence of Zebu germplasm may be the remnant of the importation of bulls used as sires for females of Creole breeds, a practice of indiscriminate crossbreeding widely used in several Latin American countries since the middle of the last century[22,33], but which, as mentioned above, is not currently common among producers in the Mixteca region. Another possible cause for the presence of Zebu germplasm may be related to the flow of ancestral genes between African Zebu cattle and Iberian cattle before they were brought to America, as has been suggested in previous studies using SNP markers and mitochondrial DNA[24,38].

The Mixteco Creole cattle show a level of genetic variability similar to that reported in studies of Creole cattle populations in the Americas. In addition, it is more genetically related to other Mexican Creole cattle populations. However, there is evidence of the influence of exotic germplasm, in smaller percentages, from specialized *Bos taurus* and *Bos indicus* breeds.

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Conflict of interests

The authors declare that they have no conflict of interests.

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