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Genetic diversity and population structure analysis of the Mexican Pastoreña Goat

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ABSTRACT

Pastoreña Goats represent an important biological and economic resource in Mexico due to their successful adaptation to the environmental conditions and the type of management exerted by the shepherds. The aim of this study was to analyze the intra-breed genetic diversity and population structure of the Pastoreña Goats from the Mixteca region in Mexico. A battery of 28 microsatellite markers and four reference populations specifically selected for their close relation to the Mexican caprine population were employed to carry out the study. Within the 28 *loci* analyzed, 203 alleles were detected, with an average of 7.250 ± 3.318 alleles per *locus*. Mean values of unbiased expected heterozygosity and observed heterozygosity were 0.689 ± 0.210 and 0.667 ± 0.213 respectively. The analysis of population structure and degree of admixture revealed that Pastoreña Goats are a well-defined population that is clearly separated from the other four reference populations used in the study. No evidence of recent genetic bottleneck in the Pastoreña Goat population was detected. The results obtained will be invaluable to establish a sustainable improvement and conservation policies of this local breed.

1. Introduction

Mexico is the second largest goat-producing country in the Americas (FAOSTAT, 2014). It has an inventory of 8,687,814 heads, which ratifies the economic importance of the goat industry in the country. An important proportion of the Mexican caprine livestock is a group of populations called Creoles. These are descendants of the interbreeding among Spanish goats brought to Mexico during the colonial times, more than 400 years ago (Montaldo et al., 2010). The significance of these populations goes therefore further from socio-economic aspects, but represent a genetic and biological resource. Pastoreña Goats are included within these creole populations and were named after transhumant goat herders, due to their capacity to travel long distances.

Pastoreña Goats are mainly distributed in rural areas of the Mixteca Region in the state of Oaxaca (Fig. 1B). Their phenotypic characteristics include: presence of horns in males and females, lack of wattles, horizontally oriented ears and a typical white coloration coat, although animals with yellow or cream color shade may also exist (Fig. 1A). As mentioned above, they are rustic animals capable of yielding under unfavorable conditions, and possess morphological characteristics like high lung capacity (determined by its thoracic perimeter), and long and strong legs which are appropriate for the long grazing periods they experience (Fuentes-Mascorro et al., 2004).

Despite their unique characteristics and socio-economic importance, the genetic potential of Pastoreña Goats is yet indeterminate. In addition, the introduction of specialized goat breeds in the area is a risk factor for this genetic resource mainly due to the absence of an adequate reproductive genetic management, which leads to indiscriminate crossbreeding or even the complete replacement of local goat populations. Therefore, it is possible that the loss of genetic variation will limit the available options to reach unpredictable future requirements, as genetic variation between breeds is not easily generated (Barker, 1994). For this reason, one of the main priorities in the development and conservation of animal genetic resources is to correctly identify local livestock populations (FAO, 2007a).

Accordingly, we sought to analyze the population structure and variability of the Pastoreña Goat breed, to obtain a genetic profile through molecular microsatellite markers. This will be very useful for

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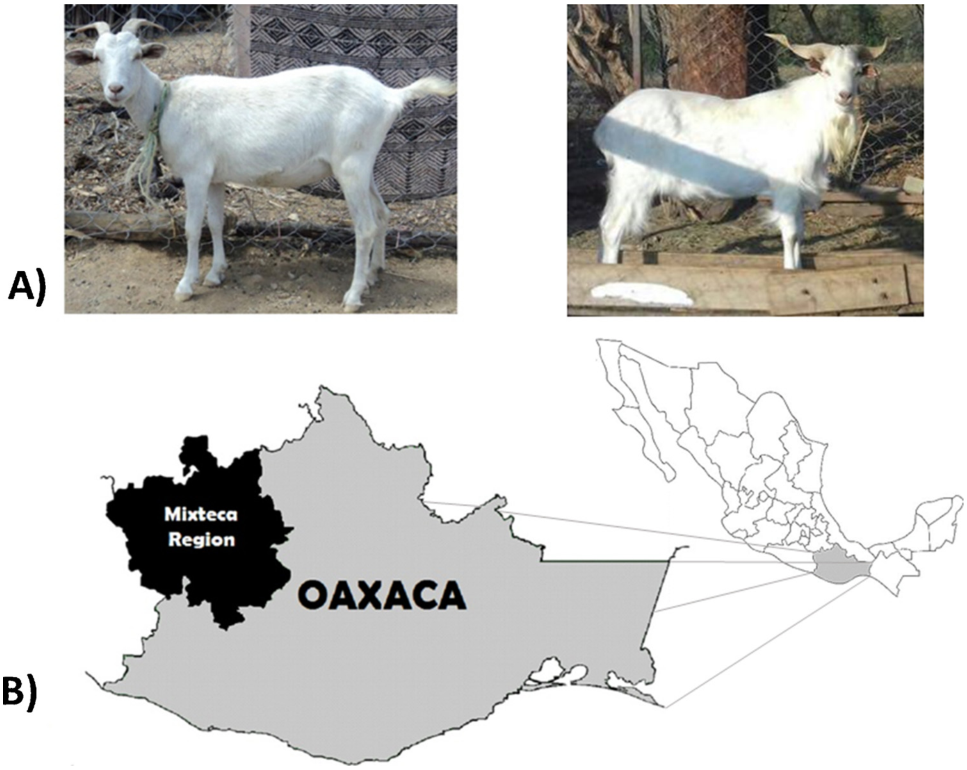

Fig. 1. A) Phenotypic appearance of a kid and adult Pastoreña Goat. B) Goegraphic location of the Mixteca Region, the main distribution área of the Pastoreña Goat population.

establishing appropriate sustainable breeding procedures and conservation policies for this local goat population.

2. Material and methods

Forty blood samples were collected from 26 females and 14 males. Sampling was performed in the municipalities of Santiago Juxtlahuaca, Tezoatlán and San Juan Mixtepec, located in the Mixteca Region of the state of Oaxaca, Mexico (Fig. 1B), which are the main breeding areas for this livestock. Pastoreña Goats were selected based on phenotypic characteristics previously reported by Fuentes-Mascorro and collaborators (Fuentes-Mascorro et al., 2011). Individuals genetically related were excluded.

DNA extraction was carried out using the ReliaPrep™ Blood gDNA Miniprep System (Promega) according to manufacturer's instructions. A battery of 28 microsatellite markers was selected from the panel recommended by FAO/ISAG (FAO, 2007b) (Table 1). Microsatellite DNA fragments were amplified as described by Martínez and collaborators (Martínez et al., 2004). The amplicons were separated by capillary electrophoresis in a Genetic Analyzer Sequencer ABI377XL (Applied Biosystems) and characterized using the internal marker Genescan 500-Liz (Applied Biosystems) and ABI Gene Mapper software (Applied Biosystems).

Genotypic data were statistically analyzed by Excel Microsatellite Toolkit (Park, 2001), Arlequin v3.1 (Excoffier and Lischer, 2010), Popgene v1.32 (Yeh et al., 1999) and Genepop (Raymond and Rousset, 1995) software. Intra-breed diversity was analyzed by calculating the following parameters of genetic differentiation: number of alleles per *locus*, effective number of alleles, allelic frequencies, expected unbiased heterozygosity, observed heterozygosity, polymorphic information content (PIC) (Botstein et al., 1980) and Shannon's information index (Lewontin, 1972). Hardy-Weinberg equilibrium deviation test (HWE) was developed by using the exact test in Popgene v1.32. Finally, correlation between gene pairs within individuals in a population (F_{IS}) was determined (Weir and Cockerham, 1984) using FSTAT software v.9.3.2 (Goudet, 1995).

The structure and genetic relationships of the Pastoreña Goat was evaluated using four goat populations (Murciano-Granadina, Blanca Andaluza, Blanca Celtiberica, Anglo-Nubian). These were selected for their historical connections with the Mexican goat populations. Murciano-Granadina, Blanca Andaluza and Blanca Celtiberica were chosen for being the Iberian origins and founding populations of Latin American goats (Mellado, 1997). On the other hand, the Anglo-Nubian population was selected because it is the main specialized goat breed introduced in the region. It is therefore important to establish the possible influence of this breed on the studied population. Reference genotypes for all populations were kindly provided by BioGoat Consortium (https://biogoat.jimdo.com/).

Nei's standard genetic distance (DST) (Nei, 1972) between the populations was estimated using the POPTREE2 software (Takezaki et al., 2010). Then, genetic distances between each pair of populations were used to construct a phylogenetic tree by using the neighbor-joining method (Saitou and Nei, 1987). A bootstrap resampling ($n = 1000$) to test the dendrogram robustness was performed. Next, a Bayesian clustering algorithm (under an admixture model) was employed to evaluate the genetic structure of the Pastoreña Goat population. This was performed by using the following parameters in STRUCTURE v2.3.4 (Pritchard et al., 2000): 200,000 burn-in; 1,000,000 Markov Chain Monte Carlo (MCMC) Iterations. Five different runs from $K = 2$ to $K = 6$ were carried out to estimate the most probable number of populations clusters. To calculate an optimal K value, the Evanno method (modal value of the distribution of the DeltaK) (Evanno et al., 2005) was implemented using the Structure Harvester Web v0.6.94 software (Earl and vonHoldt, 2012).

Finally, the possible effect of a genetic bottleneck was investigated in BOTTLENECK v.1.2.02 software (Cornuet and Luikart, 1996) using a two-phase model (TPM), with 10,000 replications. To determine if a population exhibited a significant number of *loci* with excess or