











## Exploring the genetic of three Hairless Pig breed populations in Mexico

Explorando la genética de tres poblaciones de la raza Cerdo Pelón en México

Explorando a genética de três populações da raça Porco Pelado no México

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### Animal production

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### Abstract

The objective of this research was to carry out a molecular genetic characterization of three Hairless Pig (HP) breed populations located in México in the states of Nayarit, Oaxaca and Yucatán to identify variations, selection effects and population genetic relationships. From blood samples, a total of 163 animals from three populations of HP were successfully genotyped. Genotyped SNP data was employed to compute genetic diversity, population structure and landscape genetic analysis. The parameters of minor allele frequency (MAF), observed heterozygosity ( $H_o$ ) and Wright's fixation index (FIS) pointed out that the population of Nayarit is the one with the greatest genetic diversity (MAF: 0.362,  $H_o$ : 0.336, FIS: 0.061). Principal Component Analysis (PCA) and a Neighbor-joining phylogenetic tree analysis showed diversification among the three HP populations and pointed out a closer genetic relationship between the HP populations and the Iberian pig breed. The Admixture analysis showed as well common ancestry shared from commercial and Iberian breeds in the HP populations in different gradients. For the analysis of the relationships between geographic and genetic distances, Mantel test was computed and the results described a distribution pattern of the geographical locations along the genetic distances similar to the clustering pattern of the PCA and Neighbor-joining phylogenetic tree analysis. The results obtained manage to present the HP poor conservation state and the need to establish a genetic conservation program meaning an effort to prevent the loss of a significant cultural, natural and genetic resource.

## Resumen

El objetivo de esta investigación fue realizar una caracterización genética molecular de tres poblaciones de Cerdo Pelón (CP) ubicadas en México en los estados de Nayarit, Oaxaca y Yucatán para identificar variaciones, efectos de selección y relaciones genéticas poblacionales. Se genotiparon con éxito un total de 163 animales de tres poblaciones de CP. Se emplearon datos de los SNP genotipados para calcular la diversidad genética, la estructura de la población y el análisis genético-espacial. Los parámetros de frecuencia de alelo menor (MAF), heterocigosidad observada ( $H_o$ ) y el índice de fijación de Wright (FIS) señalaron que la población de Nayarit es la de mayor diversidad genética (MAF: 0.362,  $H_o$ : 0.336, FIS: 0.061). El Análisis de Componentes Principales (ACP) y el análisis filogenético mostraron una diversificación entre las tres poblaciones de CP y señalaron una relación genética estrecha entre las poblaciones de CP y la raza de cerdo ibérico. El análisis de Admixture mostró también una ascendencia compartida de razas comerciales e ibéricas en las poblaciones de CP. En el análisis genético-espacial los resultados describieron un patrón de distribución de las ubicaciones geográficas en relación con las distancias genéticas similar al patrón de agrupamiento del ACP y del análisis del árbol filogenético. Los resultados obtenidos logran presentar el mal estado de conservación del CP y la necesidad de establecer un programa de conservación genética que signifique un esfuerzo para evitar la pérdida de un importante recurso cultural, natural y genético.

**Palabras clave:** diversidad genética, genética del paisaje, razas nativas, recursos genéticos.

## Resumo

O objetivo desta pesquisa foi realizar uma caracterização genética molecular de três populações da raça Porco Pelón (PP) localizadas no México nos estados de Nayarit, Oaxaca e Yucatán para identificar variações, efeitos de seleção e relações genéticas populacionais. Um total de 163 animais de três populações de PC foram genotipados com sucesso a partir de amostras de sangue. Dados SNP genotipados foram utilizados para calcular a diversidade genética, estrutura populacional e análise genética da paisagem. Os parâmetros de diversidade genética mostraram diferentes graus de diversidade entre as três populações de PP e indicam que a população Nayarit é a que apresenta maior diversidade genética. A Análise de Componentes Principais (ACP) e a análise filogenético de Neighbor-joining mostraram diversificação entre as três populações de PP. A análise de mistura mostrou uma ancestralidade comum compartilhada entre raças comerciais e ibéricas (grupos externos) para populações de PP em diferentes gradientes. Para analisar as relações entre distâncias geográficas e genéticas, foi realizado o teste de Mantel e os resultados descreveram um padrão de distribuição de localizações geográficas em relação às distâncias genéticas semelhante ao observado no padrão de agrupamento ACP e na análise filogenético de Neighbour-joining. Os resultados obtidos conseguem apresentar o mau estado de conservação do PP e a necessidade de estabelecer um programa de conservação genética que signifique um esforço para evitar a perda de um importante recurso cultural, natural e genético.

**Palavras-chave:** diversidade genética, genética da paisagem, raças nativas, recursos genéticos.

## Introduction

The introduction and presence of the pig in the country date back 500 years ago and there are currently local genotypes whose populations are in critical and risky conservation states, as is the case of the Hairless Pig breed (DAD-IS, 2020; Sierra *et al.*, 2016). The Hairless Pig breed (HP) can be found in the tropical areas of Mexico and its coasts, both the Gulf and the Pacific and center of the country as well, adapted to different climates and altitudes in different production systems (Lemus-Flores *et al.*, 2001), the latter influencing its genetic composition and therefore its relevance in knowing them. Its physical and morphological characteristics include visible “Iberian type” features such as its grayish black color, rectilinear head, ears that fall over the eyes, and the absence of hair (DAD-IS, 2020; Lemus-Flores *et al.*, 2020). For decades, the HP breed has had relevance in the survival economy of the Mayan people and for this reason it is considered a notable zoogenetic resource as it has great rusticity and the ability to take advantage of foods with low nutritional content effectively, in addition to being part in its traditional gastronomy (Hernández *et al.*, 2020; Rosado-Aguilar *et al.*, 2022). The HP breed in Mexico has been displaced from traditional production systems, thus decreasing its populations due to the introduction of select breeds (Sierra *et al.*, 2005). At present, there is no formal record of an estimated number of individuals along the country and in the official information of this organism there is no phenotypic, productive or molecular characterization data (DAD-IS, 2020). Despite the above, currently there are efforts to rescue it since the demand for the consumption of its products has increased, especially in the so-called gourmet market (Hernández *et al.*, 2020). By implementing current methodologies, it is possible to determine the origin, genetic diversity, ancestry of the HP breed and thereby implement strategies to know its current identity and its genetic differences with respect to other breeds to establish a racial guarantee for both the breed and its products (Govindarajan *et al.*, 2012; Kawęcka *et al.*, 2016). This research allowed, through studies of SNPs in different populations of HP breed, to carry out a study of genetic diversity and population structure that will allow us to have a historical basis for future actions of genetic assessments in support of rescue and conservation programs.

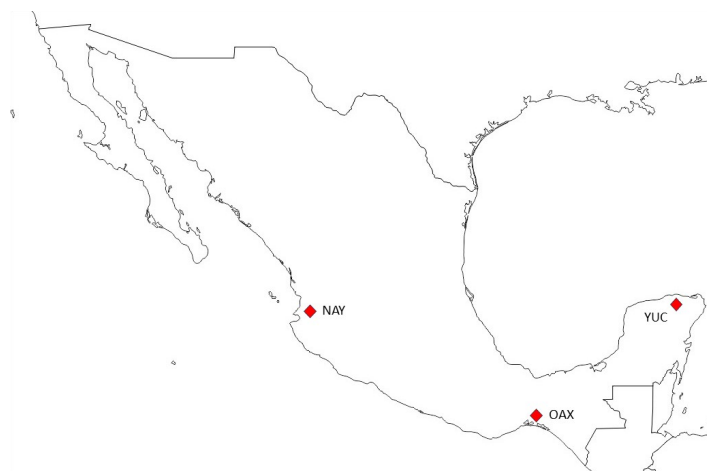
## Material and methods

### Sample collection, genotyping and quality control

This study was carried out in three populations of Hairless Pig breed (HP) defined according to the characteristics described in the study by Lemus-Flores *et al.* (2020) (absence of hair, black skin, without spots, black hoof and straight snout) located in Mexico: the northwest Pacific coast (Nayarit; 8 females and 2 males from the Faculty of Veterinary Medicine and Zootechnics of the Autonomous University from Nayarit, Compostela, semi-technified production system), the southwest Pacific coast (Oaxaca; 8 females and 2 males from the locality of San Francisco Ixhuatán, rustic production system) and southeast Atlantic coast (Yucatán; 117 females and 26 males from the Mexican Association of Iberian Origin Pig Breeders of Yucatán, Tizimín, semi-technified production system) (figure 1). Unrelated individuals were selected for this study.

Blood samples were taken from the jugular vein and collected in Vacutainer EDTA K2 tubes (Becton Dickinson, Franklin Lakes, NJ, USA) following the recommendations of the Official Standards NOM-051-ZOO-1995 on the humane treatment of animals, and NOM-062-

ZOO-1999 on the technical specifications for the production, care and processing. Later, a blood sample from each tube was collected individually in blood cards (Neogen, Nebraska, USA) and were shipped to Neogen (Neogen, Nebraska, USA) for genotyping using the Porcine GGP 50K microarray chip (Neogen, Nebraska, USA) was used for genotype analysis. A total of 163 animals from three populations of HP breed were successfully genotyped and a total of 46,145 autosomal SNPs, out of the 50698 SNPs. The quality control of data obtained from the analyzes was carried out using the PLINK v1.9 software (Chang *et al.*, 2015) where SNPs with polymorphisms with a value > 0.90 were included and considering the MAF (minor allele frequency) to a value < 0.01 were excluded.



**Figure 1. Geographic locations of the three populations of Hairless Pig described in the present study.** NAY, Faculty of Veterinary Medicine and Zootechnics of the Autonomous University of Nayarit, Compostela, Nayarit (21°13'49"N 104°52'56"W); OAX, San Francisco Ixhuatán, Oaxaca (16°20'57"N 94°28'57"W); YUC, Tizimín, Yucatán (21°08'43"N 88°08'58"W).

## Genetic diversity indices

The genetic diversity analysis was evaluated considering the parameters of minor allele frequency (MAF), observed heterozygosity (Ho), expected heterozygosity (He) and the fixation index of individuals within the populations (Fis) with the PLINK v1 software. 1.9 (Chang *et al.*, 2015).

### Genetic structure and ancestry

Principal Component Analysis (PCA) and the construction of the graphs were designed using the R software (R Core Team, 2021) to establish sample clustering projection onto the axis using the three HP breed populations (Nayarit, Oaxaca and Yucatán). In addition, publicly SNP databases from Iberian, Duroc and Large-White breeds (Burgos-Paz *et al.*, 2013; Yang *et al.*, 2017) were considered for comparisons.

Also, a Neighbor-joining phylogenetic tree was built with the DarWin software (Perrier and Jacquemoud-Collet, 2006) based on data on the genetic IBS (identity by state) distances between the studied populations. A bayesian clustering of individuals was also estimated using Admixture 1.3 (Alexander *et al.*, 2009). Herein, the K=1 to K=6 genetic groups (clusters) were tested and cross validation error was used to select the most likely K according to the author.

## Relationships between geographic and genetic distances

To determine the relationship between the geographic and genetic distances of the HP breed populations, the Mantel test was performed employing the GenAlEx 6.5 software (Peakall and Smouse, 2012) using the site coordinates of the populations and the genetic IBS distance matrix generated using PLINK v1 software 1.9 (Chang *et al.*, 2015).

## Results and discussion

### Genetic diversity

The highest values of Minor Allele Frequency (MAF) were observed for HP populations from Nayarit and Oaxaca, while that the lowest were presented by the population of Yucatán. The values of expected heterozygosity (He) were the same for the three populations, while those of observed heterozygosity (Ho) were higher in the Nayarit and Yucatán populations and lower in the Oaxaca population. The highest values for the Inbreeding Coefficient (FIS) were shown by Oaxaca population, while in contrast, the lowest were presented by the Yucatán and Nayarit populations. Similar diversity values were registered in Muñoz *et al.* (2019) and Bordonaro *et al.* (2023) in European local pig breeds where the average Ho, and He values were from 0.270 to 0.297 and 0.275 to 0.303 respectively. The above seems to indicate that the Nayarit population is the one with the greatest genetic diversity, since it presents higher levels of He and Ho, followed by the Yucatán and Oaxaca populations, presumably due to the lack of adequate management of its crossing with other commercial breeds (Meuwissen *et al.*, 2020; DeWoody *et al.*, 2021). The high FIS values along with the low heterocigocity values (He, Ho) in the Oaxaca population, suggested a considerable degree of inbreeding, since FIS measures how much excess or reduction in heterozygosity is present between observed and expected heterozygosities in a population (Li *et al.*, 2004). The above may be caused by the small number of the population, the rustic production system from which they come and the issues that it implies like poor breeding control (Šalamon *et al.*, 2019, Zhong *et al.*, 2023).

**Table1. Genetic diversity parameters of the three populations of Hairless Pig described in the present study.**

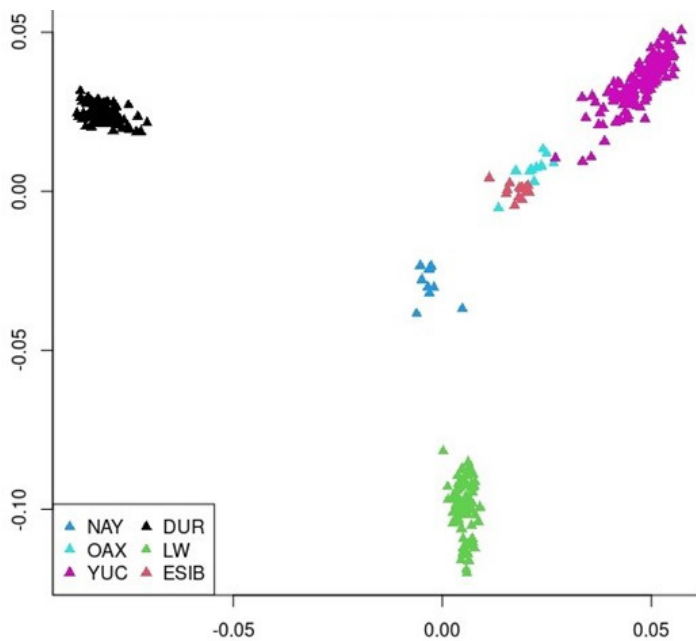
Population	N	MAF	Ho	He	FIS
Nayarit	10	0.362	0.336	0.358	0.061
Oaxaca	10	0.312	0.302	0.358	0.160
Yucatán	143	0.263	0.332	0.358	0.072

N: Sample size, MAF: minor allele frequency, Ho: observed heterozygosity, He: expected heterozygosity, FIS: Wright's fixation index, for each analyzed population.

### Genetic structure and ancestry

The grouping pattern observed in the PCA (figure 2) showed three separated clusters for the HP breed populations and another two individual clusters for the commercial breeds (Duroc and Large-White breeds) except for the Iberian breed, which overlapped with the Oaxaca HP breed population.

Also, a Neighbor-joining phylogenetic tree (figure 3) showed a close relationship for the Yucatán and Oaxaca HP breed populations are closer to the Iberian pig.



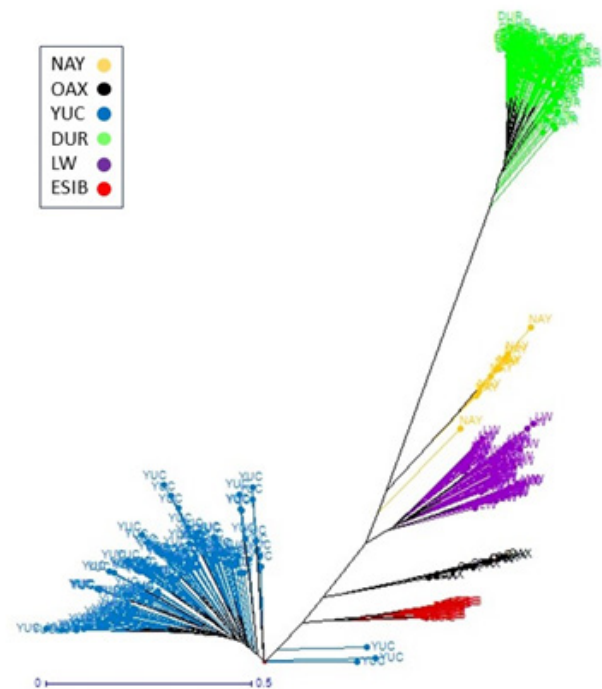
**Figure 2. Principal Component Analysis employing genetic distances and commercial pig breeds data.** NAY: Nayarit Hairless Pig breed population, OAX: Oaxaca Hairless Pig breed population, YUC: Yucatán Hairless Pig breed population, DUR: Duroc breed pig population, LW: Large-White pig breed population, ESIB: Iberian pig breed population.

This suggests a closer relationship between the Iberian breed with the population of Nayarit compared to commercial breeds (Large-White and Duroc breeds). The latter results along with the PCA data analysis, shows a similar distribution pattern in the HP breed populations. The different distribution of the genetic relationships among the commercial breeds and the HP breed populations observed in a different gradient, denote their genetic diversity possibly due to the highly variable feeding and breeding practices that can be found in native breed populations (Monteiro *et al.*, 2019), as shown in Šalamon *et al.* (2019) and Zorc *et al.* (2022) where the genetic diversity of Croatian, Serbian and Eslovenian local pig breeds results heterogeneous.

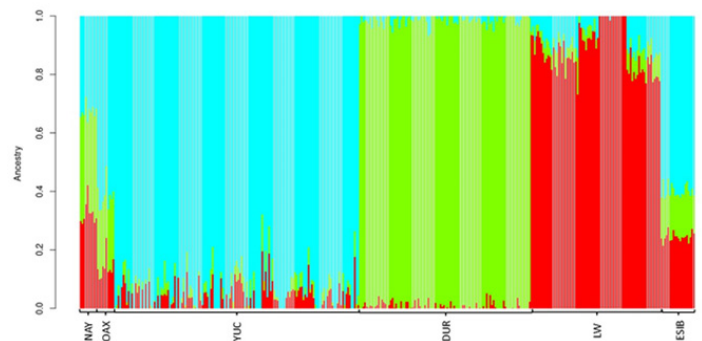
According to the results of the Admixture analysis (figure 4) ancestry by the commercial breeds can be observed in the three HP breed populations. We evaluated different K values with the mixed ancestry model (K = 1 to 6) and the one with lowest CV value was chosen. Greater ancestry was shown especially in the Nayarit population, in contrast with the Yucatán population. It can be observed that the patterns in the HP breed populations exhibit historical influence of the Iberian pig breed than to the other commercial breeds data (Duroc and Large-White), especially the Oaxaca HP breed population, agreeing with the above PCA and Neighbor-joining phylogenetic tree analysis results, the latter, in accordance with Lemus *et al.* (2023), where they founded closer genetic relationships between Iberian breeds and the HP breed.

To determine the relation between geographical and genetic distances of the HP breed populations, Mantel tests were performed in GenAIEx 6.5 software (Peakall and Smouse, 2012) employing

the populations site coordinates and IBS (Identity by state) genetic distance matrix (figure 5). The coefficient of determination ( $r^2$ ) was 0.63, suggesting a positive correlation between genetic and geographical distances.

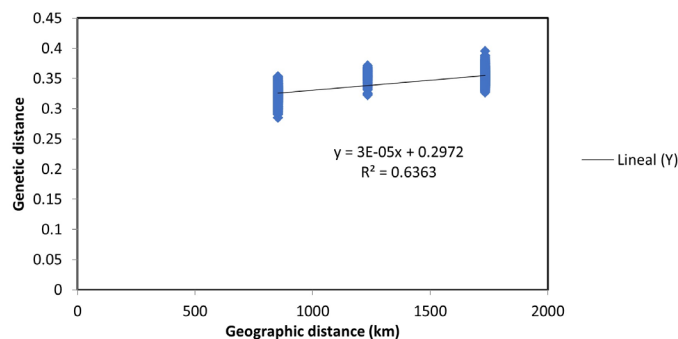


**Figure 3. Neighbor-joining phylogenetic tree in root like pattern employing IBS analysis data.** NAY: Nayarit Hairless Pig breed population, OAX: Oaxaca Hairless Pig breed population, YUC: Yucatán Hairless Pig breed population, DUR: Duroc breed pig population, LW: Large-White breed pig population, ESIB: Iberian pig population.



**Figure 4. The proportion of mixed ancestry in the populations analyzed was evaluated by ADMIXTURE 1.3 (Alexander *et al.*, 2009) with a K=3 value. We evaluated different K values with the mixed ancestry model (K = 1 to 6).** NAY: Nayarit Hairless Pig breed population, OAX: Oaxaca Hairless Pig breed population, YUC: Yucatán Hairless Pig breed population, DUR: Duroc breed pig population, LW: Large-White breed pig population, ESIB: Iberian pig population.

Through the PCA, Neighbor-joining phylogenetic tree analysis and Mantel test results, a pattern can be described in the relationship between genetic differentiation and geographical distances among the three HP breed populations.



**Figure 5. Mantel test results. A distribution pattern can be observed according to genetic distances and geographical distances between populations.**

The pattern showed greater diversity and differentiation as the spatial distribution of the three HP breed populations analyzed were further from the southeast of the country (Yucatán) and its southwest and northwest coasts (Oaxaca and Nayarit) (figure 1), drawing a gene flow presumably carried out by anthropogenic factors, the latter, possibly due to the original and first introduction points of European pig breeds in the colonization events of America during the 15<sup>th</sup> and 16<sup>th</sup> centuries carried out by the Spanish Empire (Hancock, 2022) and clearly distinguishing three distinct groups, as it is analyzed in Cesconeto *et al.* (2017) and Pimentel *et al.* (2023), where it is established a positive correlation between geographical and genetic distances in animals involved in anthropogenic activities (pigs and horses respectively) clearly differentiating them into different regional groups.

The clear genetic diversity of HP populations throughout their geographical distribution and how in different locations their genetic structure continues to be different, indicates that even belonging to the same race, the populations present great diversity between them indicating that despite their state of conservation they present levels of introgression by commercial breeds demonstrating a poor conservation state and the need to establish a genetic conservation program.

## Conclusions

The results obtained indicate that the three HP breed populations analyzed in the present research are different from each other and the genetic diversity analysis results suggest that they are closely related to the Iberian breed, genetically distant from the commercial breeds and, at the same time, share ancestry in different degrees with them. The above seems to indicate that the genetics of the HP breed are different according to its distribution throughout the country and their heterogeneous production systems.

The latter unveils their poor conservation state and the need to establish a genetic conservation program, meaning an effort to prevent the loss of a significant cultural, natural and genetic resource.

Have a more detailed description of the current state of these populations, manage to present a clearer perspective of the future necessary actions that must be taken for their adequate conservation as local breeds and, as far as possible, establish a genetic reserve program.

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