GMR
The Original by FUNPEC-RP

Genetic diversity of three indigenous pig breeds in Colombia

J.F. Rocha, R. Martínez, Y. Abuabara

Corporación Colombiana de Investigación Agropecuaria – Agrosavia.
Mosquera, Cundinamarca, Colombia

Corresponding author: J.F. Rocha
E-mail: jfmartinez@agrosavia.co

Genet. Mol. Res. 17 (4): gmr18161
Received September 28, 2018
Accepted October 26, 2018
Published October 31, 2018
DOI http://dx.doi.org/10.4238/gmr18161

ABSTRACT. Zungo (ZU), San Pedreño (SP) and Casco de Mula (CM) are Colombian indigenous pig breeds originated from European populations that are well adapted to tropical conditions. These breeds have a great potential to be used in pure and crossbred schemes due to their high reproductive performance and resistance to tropical diseases. We investigated the genetic diversity and genetic structure of these pig breeds using microsatellite molecular markers. Fifty-five ZU, SP and CM animals were genotyped for 10 microsatellites using capillary electrophoresis. The average number of alleles per marker ranged from 5.56±1.88 in ZU to 6.70±1.64 in SP. Observed heterozygosity ranged from 0.68±0.05 in ZU to 0.74±0.03 in SP. Polymorphic information content was high for most of the microsatellites in all breeds. Inbreeding coefficient (F_{IS}) values were low for all microsatellites, with an average of 0.035±0.037. Higher average values closer to and above 0.1 were observed for the fixation index of the global population (F_{IT}) (0.131±0.041) and the coefficient of relatedness between individuals from different populations (F_{ST}) (0.099±0.013). Principal components and structure analyses showed a high level of clustering per breed and very low admixture levels between them. The panel of markers used in this study proved to be useful to investigate genetic diversity and pedigree relationships in pig populations. The Colombian pig breeds that we evaluated had a high genetic variability and a well-

differentiated genetic structure, which are key for decision making in conservation programs and for the implementation of future animal breeding plans in these populations.

Key words: Zungo; Casco de Mula; San Pedreño; porcine; genetic structure

INTRODUCTION

In Colombia, there are several indigenous pig populations that originated from animals introduced by the Spaniards during the colonization period of the Americas. The Zungo (ZU) breed can be found in the Atlantic region and is characterized by having a black coat color with low hair density, floppy ears and short legs. The San Pedreño (SP) breed is mostly located in mountainous departments of the Andean region such as Antioquia, Caldas, Risaralda and Quindío. This breed has a black coat color but with abundant hair and a short snout. Finally, Casco de Mula (CM) is a pig breed mostly located in the Orioquía region (plains) of Colombia, recognized by not being cloven-hoofed. These animals are syndactyls; the two central digits on both the fore and hind feet are fused into one (FAO, 2010).In general, these three indigenous pig breeds have good reproductive performance and high resistance to parasitic diseases. They have developed physiological mechanisms that give them the ability to adapt to harsh tropical environments with high temperatures, high humidity, low food quality and multiple diseases (Oslinger et al., 2006).

Conservation and characterization of animal genetic resources is critical because of their contribution to the sustainable livelihoods of rural communities and to global food security for present and future generations (FAO, 2015). Conventional methods for assessing animal genetic resources include morphological, cytological and biochemical markers. However, characterization using molecular markers is the most reliable method available for investigating genetic variability among different populations and individuals. Hence, the rapid development of genotyping methods has permitted the use of different types of DNA polymorphisms, e.g. restriction fragment length polymorphisms, random amplified polymorphic DNA, amplified fragment length polymorphism, single-strand conformation polymorphism, microsatellite DNA and single nucleotide polymorphisms (Yang et al., 2013).

Microsatellites are a convenient first approach to genetically characterize livestock populations because of its lower cost and lower requirements regarding bioinformatics infrastructure and complexity of analytical models (FAO, 2015). Genetic diversity studies using microsatellites have been made of Brazilian (Silva et al., 2011), Uruguayan (Montenegro et al., 2015), Chinese (Jiang et al., 2015), Indian (Sahoo et al., 2016) and Croatian (Margeta et al., 2018) indigenous pig breeds. Moreover, a very comprehensive study on the conservation priorities of Ibero-American pig breeds based on microsatellite information was carried out by Cortés et al. (2016).Along the same line, we characterized the genetic diversity and genetic structure of three Colombian indigenous pig breeds using a set of 10 microsatellites.

MATERIAL AND METHODS

Animals

All the animals used in this study belong to the Sistema de Bancos de Germoplasma de la Nación para la Alimentación y la Agricultura (SBGNAA) for animal genetic resources, kept and managed by Corporación Colombiana de Investigación Agropecuaria (Agrosavia). Fifty-five animals of three different Colombian indigenous breeds were used in this analysis as follows: Zungo (ZU) (*N*=16), San Pedreño (SP) (*N*=21) and Casco de Mula (CM) (*N*=18). ZU pigs were kept in the experimental station C.I. Turipaná (Cereté, Córdoba department) of Agrosavia, while SP and CM pigs were kept in the El Nus (San Roque, Antioquia department) and La Libertad (Villavicencio, Meta department) experimental stations, respectively. Blood samples were obtained from all the pigs using collection tubes containing an anticoagulant.

DNA extraction and genotyping

DNA was extracted following a standard commercial protocol (MoBio Laboratories, Inc., Carlsbad, CA, USA). After extraction, the quality and quantity of the DNA samples were estimated using a spectrophotometer (Nanodrop 2000®, Thermo Scientific, USA) and each sample was diluted to 50 ng/µL and stored at -70°C.

PCR technique was used to amplify the following 10 microsatellites recommended by FAO for genetic molecular characterization studies in pigs (FAO, 2011): S0090, SW72, SW2406, SW24, SWr1941, SW240, S002, SW911, S0097 and SW857. Each PCR tube with a final volume of 10 µL contained 50-100 ng of DNA, 100 µM of each dNTP, 1.0 U of DNA polymerase (AmpliTaq Gold DNA Polymerase, Applied Biosystems), 4-20 µMof labeled forward primer and of unlabeled reverse primer (depending on the primer), and 50 µMof $MgCl_2$. The amplification was carried out in an iCycler (BioRad) thermocycler and the PCR cycling conditions employed were as follows: an initial denaturation step at 95°C for 10 min followed by 36 cycles of denaturation at 94°C for 75 s, annealing at 55°C or 60°C (depending on the microsatellite) for 75 s, extension at 72°C for 90 s, and a final extension at 72°C for 50 min. The fluorescent labeled PCR products were mixed with 11.5 uL Hi-Di formamide and 0.5 uL Liz 500TM internal size standard. This mix was later denatured at 95°C for 5 min, followed by a rapid thermal shock at -20°C. Finally, these samples were genotyped on a capillary electrophoresis ABI PRISM® 310 DNA analyzer (Applied Biosystems). DNA sizing and quality allele calls for all microsatellites were performed using Genemapper software 4.1 (Applied Biosystems).

Statistical analysis

Allele frequencies, observed (*Ho*) and expected (*He*) heterozygosities, F-statistics, and Hardy-Weinberg equilibrium per population were calculated using

GenePop 3.3 software (Laboratoire du Genetique et Enviroment, Monpellier, France). Genetic differentiation was examined in two steps. In the first step, the genetic distances between and within breeds were estimated using the Nei method included in the PHYLIP software (Felsenstein, 2005), with a preceding bootstrapping process that included resampling with 10,000 repetitions. In the second step, a principal component analysis (PCA) of the genetic distances was performed using the Genetix software (Laboratoire Génome, Populations et Interactions, CNRS-UMR 5000, Université de Montpellier II, Montpellier, France).

Subsequently, a Bayesian iterative algorithm was implemented to establish the genetic structure of the population using genotype data from unlinked markers. This was performed with the Structure 2.1 software (Pritchard et al., 2000), which randomly assigns individuals to a predetermined number of genetic groups K, according to the genotype of the multiple *loci* evaluated, measuring the admixture levels within individuals and determining the *K* value for the parental populations. A representative *K* value for the sample set was obtained by running four independent simulations for three preselected *K* values ($1 \leq K \leq 4$). All runs were performed with 200,000 burn-in periods and 100,000 Markov Chain Monte Carlo (MCMC) iterations after burn-in. Finally, the most appropriate *K* value in the populations was calculated with the ΔK algorithm used by Evanno et al. (2005).

RESULTS

The average number of alleles per marker ranged from 5.56±1.88 in ZU to 6.70±1.64 in SP (Table 1). The lowest values for *He* and *Ho* (0.69±0.03 and 0.68±0.05, respectively) were observed in CM and ZU pigs, respectively, whereas the highest values for both *He* and *Ho* (0.76±0.02 and 0.74±0.03, respectively) were seen in the SP breed. Overall, the largest difference between H_e and H_o was observed in ZU pigs, whereas the smallest difference was found in CM pigs. However, no large differences were observed overall between the *He* and *Ho* values for all breeds.

Table 1. Expected (H_e) and observed (H_o) heterozygosity for three Colombian indigenous pig breeds.

Breed[1]	No. Loci	*He*	*Ho*	AN[2]
CM	9	0.69±0.03	0.70±0.04	5.67±1.66
SP	10	0.76±0.02	0.74±0.03	6.70±1.64
ZU	9	0.71±0.04	0.68±0.05	5.56±1.88

[1]CM = Casco de Mula; SP = San Pedreño; ZU = Zungo

[2]AN = Mean allele number

The polymorphic information content (PIC) was high for most of the microsatellites analyzed in all breeds (Table 2). Medium PIC values were observed for SWr1941 (in ZU pigs), SW240 (in all breeds) and SW857 (in CM and ZU pigs). The lowest PIC value was observed for the microsatellite S002 (in CM pigs). F-statistics are shown in Table 3. In general, F_{IS} values were low for all microsatellites, with an average value of 0.035±0.037. These values per breed are not shown in the table, but are as follows: -0.017 for CM, 0.031 for SP and 0.047 for ZU. Higher average values closer to and above 0.1 were observed for F_{IT} (0.131±0.041) and F_{ST} (0.099±0.013).

Table 2. Polymorphic information content (PIC) for 10 microsatellites genotyped in three Colombian indigenous pig breeds.

Locus	CM	SP	ZU
S0090	0.75	0.78	0.75
SW72	0.74	0.60	0.69
SW2406	-	0.69	-
SW24	0.68	0.64	0.44
SWr1941	0.64	0.77	0.50
SW240	0.56	0.55	0.50
S002	0.44	0.76	0.73
SW911	0.71	0.74	0.81
S0097	0.59	0.74	0.77
SW857	0.52	0.74	0.52
Mean	0.63	0.70	0.63

Pig breeds: CM = Casco de Mula; SP = San Pedreño; ZU = Zungo

Table 3. F-statistics for 10 microsatellites genotyped in three Colombian indigenous pig breeds.

Microsatellite	F_{IS}	F_{IT}	F_{ST}
S0090	0.043	0.147	0.109
SW72	0.043	0.135	0.096
SW24	0.038	0.136	0.103
SWr194	0.003	0.097	0.095
SW240	0.041	0.135	0.098
S002	0.029	0.118	0.092
SW911	0.053	0.146	0.099
S0097	0.034	0.135	0.104
SW857	0.033	0.128	0.098
Mean	0.035	0.131	0.099
Standard deviation	0.037	0.041	0.013

Inbreeding coefficient (F_{IS}); Fixation index of the global population (F_{IT}); Coefficient of relatedness between individuals from different populations (F_{ST})

The PCA analysis allowed the production of a spatial representation for all the individuals included in the study, according to their genetic variability and distance (Figure 1). This analysis clearly distinguished the SP breed from the CM and the ZU breeds, with the first and second factorial axes accounting for 57 and 43% of the total genetic variation, respectively. CM and ZU breeds were located slightly closer compared to the SP breed. There were no evident substructures revealed within breeds.

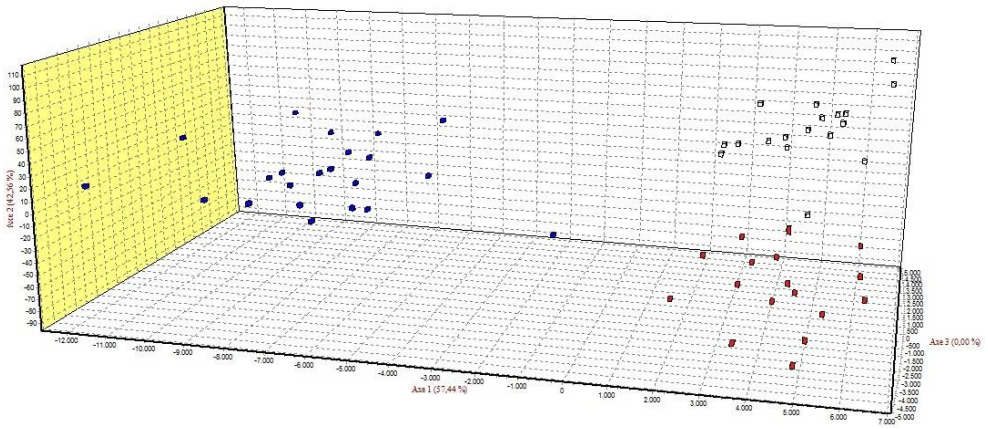

Figure 1. Principal component analysis of allele frequencies obtained from microsatellites genotyped in 55 Colombian indigenous pig breeds. Each dot in the figure represents an individual and the color denotes the breed (San Pedreño in blue, Casco de Mula in white and Zungo in red).

The analysis to explore the genetic structure of the three pig populationsshowed a high degree of differentiation among all breeds (Figure 2). The optimum number of clusters identified by the Structure software was *K = 3*. Most CM and ZN pigs (represented by red and blue areas, respectively) showed little or no evidence of introgression from the other breeds. However, some SP animals showed high admixture levels with the other breeds.

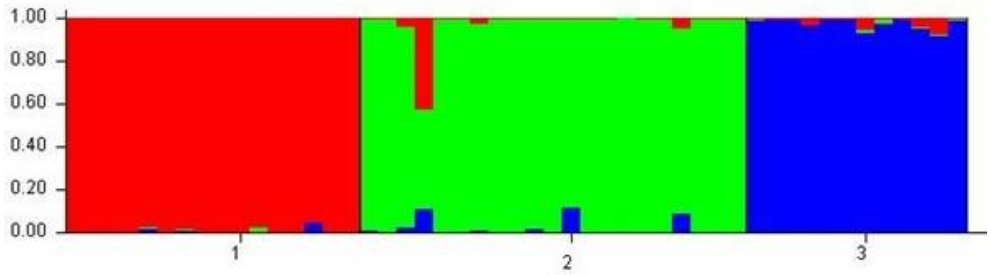

Figure 2. Inference of population genetic structure for three Colombian pig breeds (red areas correspond to Casco de Mula pigs, green areas stand for San Pedreño pigs and blue areas correspond to Zungo pigs).

DISCUSSION

Most of the *loci* were highly polymorphic. Mean allele number (AN) in Colombian pigs was similar to the AN found in specialized and locally adapted Brazilian pigs (6.18) (Silva et al., 2011), Ecuadorian creole pigs (6.2) (Vargas et al., 2016) and Uruguayan Pampa Rocha pigs (5.72) (Montenegro et al., 2015). All three Colombian pig breeds showed a higher AN than what Jiang et al. (2015) found in Chinese Laiwu pigs (4.47), but lower than the AN observed in Indian pigs (11.27±0.85) (Sahoo et al., 2016). Likewise, the mean PIC for all Colombian pig breeds was higher than that found for Uruguayan Pampa Rocha pigs (0.56) (Montenegro et al., 2015); only the SP breed showed a higher mean PIC (0.70) compared to the one found in Brazilian pig breeds (0.66) (Silva et al., 2011). Differences in AN and PIC found between these Colombian pig breeds and other pig populations from other countries might be due to the lower number of markers assessed in our study. Overall, most microsatellites in this study had a high PIC (>0.5) indicating that they are very informative for these pig populations.

The mean *He* and *Ho* values obtained for CM (0.69±0.03 and 0.70±0.04), SP (0.76±0.02 and 0.74±0.03) and ZU pigs (0.71±0.04 and 0.68±0.05) were higher than the values found in Ecuadorian creole pigs (0.66 and 0.60, respectively) (Vargas et al., 2016), and Uruguayan Pampa Rocha pigs (0.60 and 0.58, respectively) (Montenegro et al., 2015). This shows the high degree of genetic variability of these Colombian pig breeds, which can also be corroborated by comparing heterozygosity values with the comprehensive list of genetic diversity indicators made by Cortés et al. (2016) for 45 Ibero-American pig breeds. In SP and ZU pigs, *Ho* was slightly higher than *He*, indicating some degree of inbreeding within these populations. However, significant differences between *He* and *Ho* were not observed for any breed, which is expected in these populations, as they belong to conservation programs of local animal genetic resources with no selection pressure for animal breeding purposes.

The F_{IS} statistic ranged from 0.003 (SWr194) to 0.053 (SW911), with a mean for all assessed markers of 0.035±0.037. Higher F_{IS} values were found in Indian pigs (Sahoo et al., 2016) for markers such as S0101, S0226 and Sw2008 (0.28, 0.37 and 0.58, respectively), and also in the Croatian Turopolje and the Black Slavonian pig breeds (Margeta et al., 2018) for markers such as SO002, SO226, SW240, SO218, SW951 (0.43, 0.48, 0.50, 0.59 and 0.79, respectively). This might be due to a phenomenon of within-population inbreeding occurring in these Asian and European breeds. Further, in Uruguayan Pampa Rocha pigs, the markers with the highest F_{IS} were S0355 (0.26), SW24 (0.21) and S0002 (0.40) (Montenegro et al., 2015); mean while more than 10 markers used for the evaluation of genetic diversity in Brazilian pigs gave F_{IS} values ranging from 0.22 to 0.60 (Silva et al., 2011). Excess of homozygotes for most markers analyzed in this latter study might be explained by the inclusion of specialized breeds that have undergone intense selection pressure for production traits. The low F_{IS} values observed in Colombian pig breeds indicate that the panel of microsatellites used in this study have optimum levels of heterozygosity and no significant inbreeding is present in these populations. This is however an expected result, as these belong to *in vivo* germplasm banks.

The mean F_{ST} observed in this study (0.099±0.013) indicates that almost 10% of the total genetic variation is explained by differences among populations, while most (90%) of the variation corresponds to differences among individuals within populations. Similar or lower F_{ST} values were found in Brazilian (0.039) (Silva et al., 2011) and Indian pigs (0.115±0.01) (Sahoo et al., 2016), while higher values were found in Croatian (0.24) (Margeta et al., 2018), South African (0.088-0.270) (Swart et al., 2010), Korean and Chinese (0.092-0.684) (Kim et al., 2005) pig populations. Differences in F_{ST} among genetic diversity studies might be due to different panels of markers used for genotyping. Nevertheless, comparisons with the other studies lead us to suggest that lower F_{ST} values, such as those found in our study and also in other studies, are more likely to be found in indigenous or autochthonous pig breeds. Conversely, higher F_{ST} values are more common in commercial or well-specialized pig breeds, which are the ones included in the South African and Asian studies.

Nonetheless, the graphic representation of the allelic differences between and within populations shown in Figure 1 revealed that the three Colombian pig breeds are grouped in different genetic clusters. There is a high level of genetic diversity within breeds, with some individuals from different breeds being very close to each other, particularly those between CM and ZU populations. On the other hand, SP pigs are more genetically distant from the other two breeds, which is supported by the higher genetic diversity explained by the first factorial axis (57%) compared with the second one (43%). Other studies made in Croatian (Margeta et al., 2018), Korean and Chinese (Kim et al., 2005) pig populations showed more differentiated clusters obtained with PCAs, due to a lower genetic diversity within populations and to a higher genetic differentiation between those breeds.

Structure analysis (Figure 2) revealed very low admixture levels for the three Colombian pig breeds. The clustering level between breeds was high for all structures tested, with a different number of hypothesized ancestral populations ($1 \leq K \leq 4$). This genetic differentiation observed in Colombian pig breeds is higher than those observed in structure analyses made of Uruguayan (Montenegro et al., 2015) and South African pig populations (Swart et al., 2010), which might be explained by the inclusion of foreign commercial pig breeds in these studies. This factor increases the probability of these to

share a common ancestral population. Additionally, the distance among the geographical locations where the ZU, SP and CM breeds are kept and the reproductive control (e.g. no crossbreeding permitted) established by the conservation program of the Germplasm Bank for animal genetic resources operated by Agrosavia are key factors that contribute to this high genetic differentiation. The low levels of introgression of the CM and ZU pig breeds detected within a few SP individuals might be explained in part by common Iberian breeds in their genealogy such as the Extremeña for ZU and SP pigs, which were introduced to the Americas in 1525 (Oslinger et al., 2006). A common ancestor population for all the pig breeds might have been brought to the country in the first Spanish voyages that took place around 1493. This population was possibly later divided into multiple populations that were then distributed into different agroecological regions throughout Colombia. Further studies are required to assess the genetic distances of Colombian and European pig breeds to elucidate or clarify the origin and genetic connection of these indigenous pigs brought centuries ago to the Americas.

CONCLUSIONS

The Colombian ZU, SP and CM pig breeds have a high degree of genetic variability, which is evidenced by a high average number of alleles per marker and high observed heterozygosity levels and polymorphic information content. The panel of markers used in this study proved to be useful to investigate genetic diversity and pedigree relationships in pig populations. There is a well-defined genetic structure within breeds that determines solid clustering of the three breeds with very low admixture levels. These findings are key for decision making in conservation programs and for future implementation of animal breeding plans in these populations.

ACKNOWLEDGEMENTS

The authors acknowledge funding received from the Ministerio de Agricultura y Desarrollo Rural of Colombia (MADR) through the Instituto Colombiano Agropecuario (ICA) and the Corporación Colombiana de Investigación Agropecuaria (Agrosavia).

REFERENCES

Cortés O, Martinez AM, Cañon J, Sevane N, Gama LT, Ginja C, Landi V, Zaragoza P, Carolino N, Vicente A et al.(2016) Conservation priorities of Iberoamerican pig breeds and their ancestors based on microsatellite information. Heredity 117:14.

Evanno G, Regnaut S and Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14:2611-2620.

FAO (2010) Plan nacional de acción para la conservación, mejoramiento y utilización sostenible de los recursos genéticos animales de Colombia. FAO, Bogotá, D.C. 180 pp.

FAO (2011) Molecular genetic characterization of animal genetic resources.FAO Animal Production and Health Guidelines. FAO, Rome.

FAO (2015) The second report on the state of the world's animal genetic resources for food and agriculture. In: Scherf BD and Pilling D (eds). FAO Commission on Genetic Resources for Food and Agriculture assessments. FAO, Rome.

Felsenstein J (2005) PHYLIP (Phylogeny Inference Package) version 3.6. Department of Genome Sciences, University of Washington, Seattle.

Jiang W, Wang Y, Xing J and Jiang Y (2015) Evaluation of the genetic diversity of Laiwu pigs using twenty-seven microsatellite markers. Biochem Syst Ecol 62:1-5.

Kim TH, Kim KS, Choi BH, Yoon DH, Jang GW, Lee KT, Chung HY, Lee HY, Park HS and Lee JW (2005) Genetic structure of pig breeds from Korea and China using microsatellite loci analysis. J Anim Sci 83:2255-2263.

Margeta P, Gvozdanović K, DjurkinKušec I, Radišić Ž, Kušec G and Margeta V (2018) Genetic analysis of Croatian autochthonous pig breeds based on microsatellite markers. Arch Zootec 67:13-16.

Montenegro M, Llambí S, Castro G, Barlocco N, Vadell A, Landi V, Delgado JV, and Martínez A (2015) Genetic characterization of Uruguayan Pampa Rocha pigs with microsatellite markers. Genet Mol Biol 38:48-54.

Oslinger A, Muñoz JE, Álvarez LA, Ariza F, Moreno F, and Posso A (2006) Caracterización de cerdos criollos colombianos mediante la técnica molecular RAMs. Acta Agron 55:45-52.

Pritchard JK, Stephens M, and Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Sahoo NR, Nesa N, Naskar S, Banik S, Pankaj PK, and Sahoo M (2016) Microsatellite and mitochondrial diversity analysis of native pigs of Indo-Burma biodiversity hotspot. Anim Biotechnol 27:52-59.

Silva ECd, Dutra Junior WM, Ianella P, Gomes Filho MA, Oliveira CJPd, Ferreira DNdM, Caetano AR and Paiva SR (2011) Patterns of genetic diversity of local pig populations in the State of Pernambuco, Brazil. Rev Bras Zootecn 40:1691-1699.

Swart H, Kotze A, Olivier PAS, and Grobler JP (2010) Microsatellite-based characterization of Southern African domestic pigs. S Afr J Anim Sci 40:121-132.

Vargas JC, Velásquez FJ and Chacón E (2016) Estructura genética y caracterización molecular delcerdocriollo (Susscrofadomestica) de Ecuador, utilizando marcadores microsatélites. Acta Agron 65(3):292-297.

Yang W, Kang X, Yang Q, Lin Y and Fang M (2013) Review on the development of genotyping methods for assessing farm animal diversity. J Anim Sci Biotechno 4:1-6.