Nigerian J. Anim. Sci. 2023, 25 (2): 12-20

Genetic diversity and phylogenetic relationships of Nigerian indigenous pigs in Southwestern Environment of Nigeria using microsatellite loci

Oluwole Olufunke[1], Okoth Edward[2], Adeola Adeniyi Charles[3], Moses Ogugo[2]

[1]Institute of Agricultural Research and Training, Obafemi Awolowo University, P. M. B. 5029, Moor Plantation, Ibadan, Nigeria
[2]Bioscieences for Central Africa, International Livestock Research Institute, Nairobi Kenya.
[3]State Key Laboratory of Genetic Resources and Evolution & Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China.

***Corresponding Author:** oluwoleolufunke@gmail.com* ***Phone Number:** +234(0)8037422069*

***Target Audience:** Researchers, Pig scientists, Geneticists, Animal breeders*

Abstract

Indigenous pigs in Nigeria are hardy and resistant to local harsh environmental conditions. This study investigated the genetic diversity and phylogeny of Nigerian indigenous pig (NIP) from three locations in Southwestern Nigeria including Ilaramokin (n = 13), Igangan (n = 5), and Igbara-Odo (n = 7) using eight microsatellite markers. The overall mean of number of alleles (Na) was 2.63±0.27 with effective number of allele (Ne) mean of 1.94±0.17. The SW 122 marker had the highest Na of 4.67±0.67 with Ne of 3.24±0.67; 0.70±0.05 and 0.67±0.06 for Ho (observed heterozygosity) and He (Expected Heterozygosity) respectively. The mean estimate 2.30±0.60 of Nm (gene flow) showed low gene flow between the three populations. The overall mean of F statistics parameters of genetic differentiation estimated were 0.06±0.07, 0.11±0.60 and 0.17±0.06 for FIS (within population inbreeding estimate,), FST (estimate of population differentiation) and FIT (overall global heterozygote deficit across population) respectively. The principal coordinate plot showed separate clustering of the three NIP populations. The phylogenetic tree showed common ancestry and separation due to geographic locations for the three NIP populations. Genetic distance showed closer relationship between the Ilaramokin and Igbara-Odo NIPs. These results will be useful in genetic improvement of the remaining pure NIP populations.

***Keywords:** Nigerian indigenous pigs; microsatellite markers; heterozygosity; genetic distance; phylogenetic tree, Molecular genetics*

Description of problems

Nigerian indigenous pigs (NIP) thrive well under a wide range of ecological conditions (1). (2) reported that Nigeria has the highest population of pigs in Africa, and the pigs accounted for about 4.45% of the total supply of meat in the country. Pigs have advantage over other animals by having a high rate of productivity of 9.3 live piglets per sow (3).

Several studies on molecular work have been done on the exotic pig even up to the genomic level (4) but few on NIP. They have been characterized cytogenetically (5; 6), serologically (7) and morphologically (8; 9; 10). Other studies have investigated the ancestry of NIP including Oluwole *et al.* (11) that unveiled their relationship with the Asian pigs. (12) further revealed that NIP have both the West (Europe/North Africa) and East/Southeast Asian ancestries.

The current practice of extensive crossing of NIP with exotic breeds to improve the size and litter traits has threatened their gene pool. NIPs are well known for their special traits such as resistance to common diseases and parasites, heat and stress tolerant, good mothering ability, and low mortality rate at birth and ability to thrive, survive and reproduce under poor management (13). Hence, they have been naturally selected under very severe environmental conditions for the last couple of decades (14). (15) has also reported the presence of African swine fever virus (ASFV) in NIP using molecular technique, polymerase chain reaction diagnosis of ASFV. They have the virus but showed no clinical symptoms of the disease (15). These traits could be introgressed into the exotic pigs to develop new breed of pig in the future such as NIGERHYBS (2). There was a steady decline in NIP, threatened with a loss of their genetic diversity through unsustainable farming practices and suggested conservation of the germplasm of this valuable genetic resource for genetic improvement (7). Number of NIP in the country is gradually decreasing and going into extinction. Therefore, there is need to preserve them (2); (5); (8); (9).

The most utilized DNA marker for population genetics of livestock is microsatellite. Microsatellite markers, also called short tandem repeats (STRs) or simple sequence repeats (SSRs), are a relatively new class of genetic marker. Over a few years they have become a tool of choice to address population genetics and demographic questions (16). The application of microsatellite markers is considered to be useful in the analysis of genetic diversity as they are numerous, randomly distributed in the genome, highly polymorphic, and show codominant mode of inheritance (17). They allow the study of genetic diversity and differentiation of closely related populations. Evaluation of performance of animals constitutes an essential part of successful breeding plans for sustainable genetic improvement. They have been proved to be one of the most powerful tools for evaluating genetic diversity and estimating genetic distances among related populations of ruminant species (18; 19; 17). Microsatellite markers have been used to analyze the genetic variation in cattle, sheep, pig, goats, buffaloes, chickens, horses, and human beings (20; 21; 22; 23; 24; 25; 26). One of the recent studies based on microsatellite markers revealed the genetic diversity and phylogenetic relationships within indigenous pigs in southern Benin (27).

Nigerian indigenous pig has not been genetically classified using molecular markers such as microsatellite markers. Factors such as the levels of inbreeding, population size, the history or origin of the breeding population, the level of selection pressure and the rate of mutation and husbandry practices affect the genetic diversity of populations. Therefore, this study aims to assess the genetic diversity and phylogenetic relationship in three populations of NIPs from Southwestern Nigeria.

Materials and Methods

DNA was extracted from 60 samples of Nigerian pigs and quantified using Nanodrops® according to the method done by (28). Genotyping analysis was done on twenty-five pure Nigerian Indigenous Pigs (NIP) from three locations (Ilaramokin (n = 13), Igangan (n = 05), and Igbara-Odo (n = 07) in Southwestern Nigeria for diversity studies within this pure breed. Eight microsatellite markers were employed using optimized primers recommended by

ISAG/FAO (2011). Polymerase chain reaction (PCR) of 15µl volume each containing of 2 µl of genomic DNA, 8.25 µl of primer mix, 1.5 µl of 10XPCR buffer (10mM Tris-HCl, 50mM.The KCl, 1.5mM MgCl2, pH 8.3), 1.2µl dNTPs (2.5 mM), 0.4µl Taq polymerase (10 unit/µl) was used. Multiplex PCR reaction was done with initial denaturation at 95°C for 15 min; 5 cycles at 94°C for 1min; 55°C for 1min; 72°C for 1min,10 cycles at 94°C for 1min; 54°C for 1:15min; 72°C for 1 min and 24 cycles at 94°C for 1 min; 53°C for 1:15min; 72°C for 1min and a final elongation at 65°C for 30min. The amplified products were checked on 1 % agarose gel and stained with green die (0.5µl). Genotyping was done using GeneMapper® Software Version 4.0 for scoring of bands.

Table 1: Analysis of molecular variance in the three populations

Source	df	SS	MS	Est.Var.	%
Among Pops	2	14.527	7.264	0.230	9%
Among Indiv	22	82.573	3.753	1.327	50%
Within Indiv	25	27.500	1.100	1.100	41%
Total	49	124.600		2.656	100%

Df- Degree of freedom; SS- ;MS Mean Square; EST. Var- Estimated Variance; %- Percentage

Table 2: Genetic diversity parameters of 8 microsatellite markers

Locus	Chr	N_a	N_e	H_o	H_e	F_{IS}	F_{IT}	F_{ST}	Nm
S01001	9	1.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	-	-	-	-
S026	16	2.33±0.33	1.67±0.16	0.47±0.07	0.39±0.06	-0.10	-0.13	0.06	3.801
S009	12	2.33±0.67	1.87±0.48	0.40±0.25	0.37±0.19	-0.07	0.1	0.16	1.324
SW24	17	3.33±0.33	2.63±0.31	0.56±0.16	0.61±0.05	0.09	0.20	0.12	1.864
SW122	6	4.67±0.67	3.24±0.67	0.70±0.05	0.67±0.06	-0.05	0.08	0.13	1.676
S0226	4	2.00±0.58	1.67±0.35	0.30±0.17	0.33±0.17	0.01	0.27	0.20	1.022
S0227	7	2.33±0.33	1.37±0.13	0.15±0.09	0.26±0.07	0.43	0.46	0.06	3.835
SW632	8	3.00±1.00	2.09±0.26	0.44±0.07	0.51±0.06	0.12	0.17	0.05	4.859
MEAN		2.63±0.27	1.94±0.17	0.38±0.06	0.39±0.05	0.06±0.07	0.17±0.06	0.11±0.60	2.30±0.60

Chr = chromosome, Na = number of alleles, Ne = number of effective alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{IS} = inbreeding coefficient, F_{ST} = fixation index, F_{IT} = overall fixation index, Nm = gene flow

** = significant ($P < 0.05$)*

Data Analysis

The GenAlEX® software version 6.41 was used to estimate allele frequencies, mean number of alleles per locus (NA), observed heterozygosity (Ho), heterozygosity expected (He) from Hardy-Weinberg (HW) assumptions. The GenAlEX® manual was fully followed. The genetic variation among population and individual of the total genetic variance were calculated with Analysis of Molecular Variance (AMOVA). The phylogenetic tree was constructed using Poptree2® software.

Results and Discussion

The analysis of molecular variance in the three populations is presented in Table 1. The result showed 9% variance among population, 50% variance among the individuals in the populations and 41% variance within individuals. These results indicated that there was no random breeding system between the animals in the study area.

From thirty-one alleles obtained from eight microsatellite markers within the three populations, all the loci were polymorphic except for S01001 that was monomorphic. The means of all the genetic parameters within the three populations is shown in Table 2. The results indicated the overall mean of number of alleles (N_a) was 2.63±0.27 with effective number of allele (Ne) mean of 1.94±0.17). The SW 122 marker had the highest N_a of 4.67±0.67 with Ne of 3.24±0.67; 0.70±0.05 and 0.67±0.06 for H_o (observed heterozygosity) and H_e (Expected Heterozygosity) respectively. The N_a obtained from this study is a little higher than the value (2.50) reported by (29) for Chinese indigenous pigs; lower than the values obtained for Ghanaian indigenous pigs (7.65) (30), Chinese pigs (4.30 – 6.10) (31; 32) and Indian indigenous pigs (7.00-7.20) (33). The mean 2.30±0.60 estimate of N_m (gene flow) showed slight gene flow between the three populations. This low gene flow might have not prevented genetic drift, thus enabling the gene differentiation between populations.

Table 3: Inter Population Genetic Diversity

Population	N_a	N_e	P_a	H_o	H_e	I
Igangan	2.25±0.59	1.83±0.42	3	0.22±0.10	0.31±0.10	0.53±0.20
Igbara-odo	3.00±0.46	2.09±0.28	6	0.44±0.09	0.44±0.09	0.78±0.17
Ilaramokin	2.63±0.32	1.91±0.20	1	0.48±0.10	0.43±0.07	0.71±0.12
Mean	2.63±0.27	1.94±0.17	5	0.38±0.06	0.39±0.05	0.67±0.09

Mean number of alleles (N_a), number of effective alleles (N_e), observed heterozygosity (H_o), number of private alleles (P_a), expected heterozygosity (H_e) and Shanon Index (I) of all 8 loci per population

Table 4 Pairwise population of f_{st} values

Igangan	Igbara Odo	Ilaramokin	
0.00			Igangan
0.10	0.00		Igbara Odo
0.12	0.05	0.00	Ilaramokin

The overall mean of F statistics parameters pertaining to genetic differentiation estimated were 0.06±0.07, 0.17±0.06 and 0.11±0.60 for within population inbreeding estimate (F_{IS}), total inbreeding estimate (F_{IT}) and estimate for population differentiation (F_{ST}) respectively. This indicates an overall heterozygosity deficiency of only 6% and a mean of 11% F_{ST} were observed between the three populations.

The inter population genetic diversity is presented in Table 3. The result showed the overall mean observed heterozygosity was lower than the expected heterozygosity except for Ilaramokin with H_o greater than $H_{e.}$. This excess heterozygosity might have been because of high technical artefacts due to the presence of null alleles present because it appears as false homozygotes (34). Similar suggestions have earlier been reported by (30) and (35), that occasional failure of alleles amplification has led to null alleles. These failures cause spurious heterozygote deficits which is a known difficulty with microsatellite markers. The same trend observed in Na and Ne of the NIP, was observed with black Slavonian pig and the Chinese indigenous pigs (29).

Table 5: Pairwise population matrix of nei genetic distance

Igangan	Igbarado	Ilaramokin	
0.00	0.88	0.85	Igangan
0.12	0.00	0.91	Igbara Odo
0.16	0.10	0.00	Ilaramokin

Note: Upper diagonal - Genetic Identity, Lower diagonal - Genetic Distance

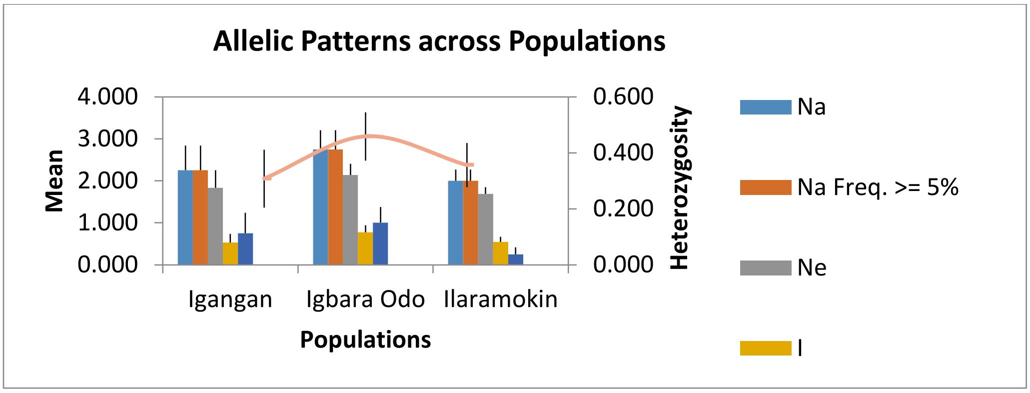

Figure 1: Allele frequency of the three population

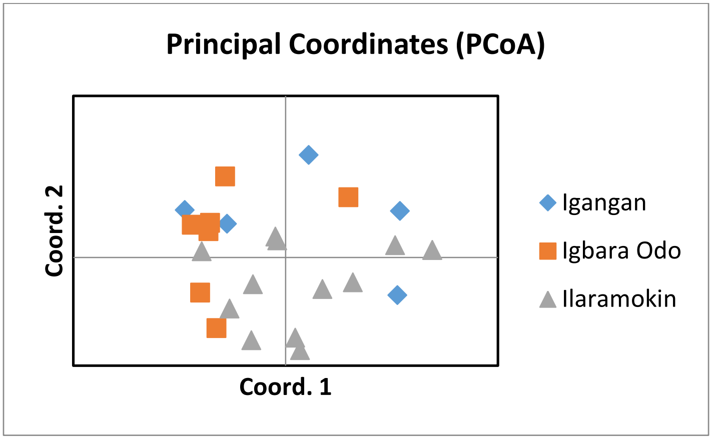

Figure 2. A scatter plot of principal coordinate analyses of pure NIP populations based on eight microsatellite markers

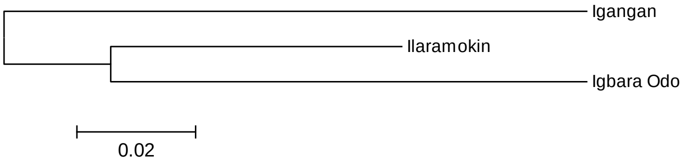

Figure 3. Neighbor-joining phylogenetic tree of pure NIP from three locations using eight microsatellites

Igbara-Odo had the highest $N_{a,}$ N_e and P_a while the mean for private alleles was 5 where Igbara-Odo had the highest private alleles of 6 . The overall mean of Shannon information index (I) was 0.67±0.09 that measures the genetic diversity of the three populations. The number of alleles (Na), effective number of alleles (Ne) and heterozygosity are presented graphically in Figure 1 for comparative visualization.

Table 4 showed the pairwise F_{ST} values among the three populations ranging from 0.05 to 0.12. The low pairwise F_{ST} values among breeds studied indicated that 95.0% to 88.0% of the total genetic variation was explained by the individual variability. These values are almost in corroboration with mean value of F_{ST} (0.11) results obtained above.

Table 5 depicted the Pairwise Population Matrix of Nei Genetic Distance (below diagonal) and Pairwise Population Matrix of Nei Genetic Identity (above diagonal) among the three pig populations. The genetic similar coefficients varied from 0.85 to 0.91 with an average of 0.88. The Genetic distances between the three pigs were 0.12 (Igangan and Igbara-Odo), 0.16 (Igangan and Ilaramokin) and 0.10 (Ilaramokin and Igbara-Odo) respectively. The Landrace and LargeWhite were closer compared with the crosses.

Figure 2 shows the outcome of the PCA conducted among the three pig populations in this study. The principal coordinate plot showed clear clustering of individual pure NIP in 3 populations. Percentage of variation explained by the first 3 axes were in 34.41, 48.42 and 60.17 percentages respectively. The Ilaramokin PCA dispersed well in all the coordinates as seen in Figure 2.

Phylogenetic relationships between populations

The phylogenetic relationship of the pigs was constructed based on Nei's standard genetic distances calculated between the NIP from 3 populations as revealed in Figure 3.. A neighbor-joining with 1000 bootstrap resampling that the branch lengths were proportional to genetic distances of the POPTREE2 software (http://www. med.kagawau.ac.jp/~genomelb/takezaki/poptree2/download.html#download). This was done by using the POPTREE manual guide. The phylogenetic trees showed that all the pure NIP originated from the same ancestor. The genetic distance relationship between the pigs revealed a close relationship between the Ilaramokin and Igbara-Odo populations. It also separated them by geographic locations with the Ilaramokin and Igbara-Odo clustering together due to closer proximity, both from Ondo state in the Southwestern Nigeria while the Igangan was from Oyo state in Southwestern Nigeria.

Conclusion and Application

In conclusion:

1. There is small genetic variances observed in the three populations of NIPs with 11.00% moderate amount of genetic diversity between them with considerable amount of inbreeding (6%) within these populations and 17% for total inbreeding estimate.
2. The phylogenetic distance revealed a common ancestry and separation due to different geographic locations.
3. The results obtained from this study will be useful in genetic improvement of the pure NIP. Therefore, our study recommends further genomic investigation on NIPs to establish more genetic potentials.
4. This study gives base-line information on the genetic improvement of NIP for proper breeding plan in the future.

Acknowledgement

"The laboratory aspects of this project was partly/fully supported by the BecA-ILRI Hub through the Africa Biosciences Challenge Fund (ABCF) program and Africa women in Agriculture and development (AWARD). The ABCF Program was funded by the Australian Department for Foreign Affairs and Trade (DFAT) through the BecA-CSIRO partnership; the Syngenta Foundation for Sustainable Agriculture (SFSA); the Bill & Melinda Gates Foundation (BMGF); the UK Department for International Development (DFID) and the Swedish International Development Cooperation Agency (Sida)."

References

1. Balogun, T. F. (1981). Swine production in Nigeria. Problems and prospects. *The Nigerian Journal of Agricultural Extension*, 4(6), 32–37.
2. Adebambo, O. A. (2003). Animal breeds: A nation's heritage. Inaugural lecture, Federal University of Agriculture, Abeokuta. Pp. 20-40.
3. C.T.A. (1995). Agricultural Extension in Africa. Technical Centre for Agriculture and Rural Cooperation. Yaounde Cameron.
4. Omitogun, O. G. (2004). Analysis of swine genome organization. Evolution of G-T-G bands in porcine chromosomes for physical mapping. ***In****: (Oriyo, O.J. Ikeobi, C.O.N. Ozoje, M.O. Omoniyi, T. and Kehinde, O.B. Eds.). Proceedings of the 29th Annual Conference of the Genetics Society of Nigeria.,* NACGRAB, Moor Plantation, Ibadan. 54–57.
5. Oluwole, O. O. (2005). Cytogenetic characterization of the Nigerian Indigenous Pig. MSc. Thesis, Obafemi Awolowo University, Ile-Ife, pg. 48.
6. Oluwole, O. O. and Omitogun, O. G. (2009). Cytogenetic characterization of Nigerian Indigenous Pig. *African journal of Biotechnology*, 8(18), 4696–4701.
7. Adeola, A. C. and Omitogun O. G. (2012). Characterization of Indigenous Pigs in Southwestern Nigeria using blood protein polymorphism. *Animal Genetic Resources*, 51, 125–130.
8. Adeola, A. C., Oseni, S.O. and Omitogun, O. G. (2013). Morphological characterization of indigenous and crossbred pigs in rural and peri-urban areas of Southwestern Nigeria. *Open Journal of Animal Sciences*, 3(3), 230–235.
9. Oluwole, O. O. (2014). Morphometric, Haematological and DNA Studies of Nigerian Indigenous Pigs, its Hybrid and Backcross in an African Swine Fever-Prone Environment. Obafemi Awolowo University. (Ph.D Thesis).
10. Yakubu, A., Durven, G.L. and Hagan, J. (2022). Multivariate analysis of body weight, morphometric and thermo-physiological traits of indigenous pigs under tropical conditions. *Genetics and Biodiversity Journal*, 6(1), 91–104.
11. Oluwole O. O., Bayene D., Okoth E., Roger, P. and Omitogun G. O. (2016). Comparative Genomics of Swine Leukocyte Antigen Class II Exons 2 and 3 of Nigerian Pigs. In Proceedings of the 33rd Biennial Conference of the Ghana Society of Animal Production. Pp. 289–293.
12. Adeola, A. C., Oluwole, O. O., Oladele, B. M., Olorungbounmi, T. O., Boladuro, B., Olaogun, S. C., Nneji, L. M., Sanke, O. J., Dawuda, P. M., Omitogun, O. G., Frantz, L., Murphy, R. W., Xie, H. B., Peng, M. S., & Zhang, Y. P. (2017). Analysis of the genetic variation in mitochondrial DNA, Y-chromosome sequences, and

MC1R sheds light on the ancestry of Nigerian indigenous pigs. *Genetics, selection, evolution*, 49(1), 52.

13. Ilori, J. A. (1974): Assessing the productive potentials of local breeds of pigs (Nigeria). *Annual Conference, Nigeria Society. of Animal Production* 1:100.
14. Adebambo, O. A. (2003). Animal breeds: A nation's heritage. Inaugural lecture, Federal University of Agriculture, Abeokuta. Pp. 20-40.
15. Oluwole, O. O and Omitogun, O. G. (2014). Polymerase Chain Reaction Detection of ASFV in Nigerian Indigenous Pigs. *America Journal of Molecular Biology*, 4(3), 159–162. http://dx.doi.org/10.4236/ajmb.
16. Ellegren, H., Moore, S., Robinson, N., Byrne, K., Ward, W., & Sheldon, B. C. Microsatellite evolution--a reciprocal study of repeat lengths at homologous loci in cattle and sheep. *Molecular biology and evolution*, 14(8), 854– 860.
17. Moore, S. S., Sargeant, L. L., King, T. J., Mattick, J. S., Georges, M., & Hetzel, D. (1991). The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics*, 10(3), 654–660.
18. Buchanan, F. C., Adams, L. J., Littlejohn, R. P., Maddox, J. F., & Crawford, A. M. (1994). Determination of evolutionary relationships among sheep breeds using microsatellites. *Genomics*, 22(2), 397–403.
19. Bowcock, A. M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J. R. and Cavalli-Sforza, L. L. (1994). High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, 368(6470), 455–457.
20. Wimmers, K., Ponsuksili, S., Hardge, T., Valle-Zarate, A., Mathur, P. K., and Horst, P. (2000). Genetic distinctness of African, Asian and South American local chickens. *Animal genetics*, 31(3), 159–165.
21. Solis, A., Jugo, B. M., Mériaux, J. C., Iriondo, M., Mazón, L. I., Aguirre, A. I., Vicario, A., and Estomba, A. (2005). Genetic diversity within and among four South European native horse breeds based on microsatellite DNA analysis: implications for conservation. *The Journal of heredity*, 96(6), 670–678.
22. Vicente, A. A., Carolino, M. I., Sousa, M. C., Ginja, C., Silva, F. S., Martinez, A. M., Vega-Pla, J. L., Carolino, N., & Gama, L. T. (2008). Genetic diversity in native and commercial breeds of pigs in Portugal assessed by microsatellites. *Journal of animal science*, 86(10), 2496–2507.
23. Serrano, M., Calvo, J. H., Martínez, M., Marcos-Carcavilla, A., Cuevas, J., González, C., Jurado, J. J., & de Tejada, P. D. (2009). Microsatellite based genetic diversity and population structure of the endangered Spanish Guadarrama goat breed. *BMC genetics*, 10, 61.
24. Hoda A., Dobi, P and Hyka, G (2009). Genetic diversity and distances of Albanian local sheep breeds using microsatellite markers. *Livestock Research for Rural Development*, 21(6), 93.
25. Jyoti Joshi1, Raj Kumar Salar1, Priyanka Banerjee, Upasna Sharma, Madhu Sudan Tantia, Ramesh Kumar Vijh. (2012). Comparative evaluation of Murrah breeds with buffaloes of Indo-Gangetic Plains. *DHR International Journal of Biomedical and Life Sciences (DHR-IJBLS)* ISSN:

2278-8301, 3(1), http://www.doublehelixresearch.com/DHRIJBLS © Double Helix Research 93.

26. Djimènou, D., Adoukonou-Sagbadja, H., Dayo, G. K., Chrysostome, C., & Koudande, D. O. (2021). Genetic diversity and phylogenetic relationships within local pigs in southern Benin. *Tropical animal health and production*, 53(4), 434
27. Oluwole O. O., Bayene D., Okoth E., Roger, P. and Omitogun G. O. (2016). Comparative Genomics of Swine Leukocyte Antigen Class II Exons 2 and 3 of Nigerian Pigs. In Proceedings of the 33rd Biennial Conference of the Ghana Society of Animal Production. Pp. 289–293.
28. Bradic M, Uremovic M, Mioc B, Konjacic M, Lukovic Z and Safner T. (2007). Microsatellite analysis of the genetic diversity in the black Slavonian pig. *Acta Veterinaria (Beograd)*, 57(2-3), 209–215.
29. Ayizanga R A, Kayang B B, Adomako K, Adenyo C, Inoue-Murayama M and Asamoah L 2016: Genetic diversity of some Ghanaian pigs based on microsatellite markers. *Livestock Research for Rural Development. Volume 28, Article #24.* Retrieved May 19, 2023, from http://www.lrrd.org/lrrd28/2/ayiz28024.html
30. Li K., Chen Y., Moran C., Fan B., Zhao S.H., Peng Z.Z., Analysis of diversity and genetic relationships between four Chinese indigenous pig breeds and one Australian commercial pig breed, Anim. Genet. 31 (2000) 322–325.
31. Behl R, Kaul R, Sheoran N, Behl J, Tantia M S and Vijh R K 2002 Genetic identity of two Indian pig types using microsatellite markers. Animal Genetics. 33: 158 – 159.
32. San Cristobal M, Chevalet C, Foulley J and Ollivier L (2003). Some methods for analysing genetic marker data in a biodiversity setting – example of the PigBioDiv data. Archivos de Zootecnia. 52: 173 – 183.
33. Chakraborty R, De Andrade M, Daiger SP, Budowle B. (1992). Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. Ann Hum Genet. 56 (Pt 1):45–57.