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ANALYSIS OF GENE TIC DIVERSITY IN MUSCOVY DUCK OF BARUTEN LOCAL GOVERNMENT KWARA STATE NIGERIA USING THE NUCLEAR CYP2U1

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ABSTRACT

The analysis of Genetic diversity within and between populations is important for development of sustainable livestock production. Muscovy duck (*Carina Moschata*) is important among the poultry specie raised by most peasant farmers in Baruten local government area of Kwara State, Nigeria for egg and meat production. This study evaluated the genetic diversity between Muscovy duck populations in some selected locations within the local government; [*Bani (BON), Okuta (OKE), Yembeleku (BART)* and *Ilesha Baruba* (ILE)] using the nuclear DNA Cytochrome P450 family 2 Subfamily U Polypeptide 1 (CYP2U1) genes. The results showed 722 monomorphic sites and 25 polymorphic sites. The sequences obtained were grouped into nine haplotypes with diversity of 0.771 and low nucleotide diversity per site (Pi=0.00608). A total number of 26 mutations were discovered with haplotype diversity ranging from 0.80 to 0.86 for ILE, BART and BON Muscovy duck. The Jalview results from 0 to 167 bp revealed some of the polymorphism consisting of substitutions and insertions in BON, BART and ILE at 33, 37, 47, 113 and 159 bp; while the phylogenetic tree showed a high level of monophyletic relationship between some of the Muscovy duck. Our study reveals high haplotype diversity but low nucleotide diversity within and between the Muscovy duck studied in Baruten local government of Kwara State, Nigeria. The high haplotype diversity is in accordance with the high number of haplotypes observed in each location, while the low nucleotide diversity reflects that Nigeria Muscovy duck are likely a descent of common maternal lineage. This finding serves as baseline information, thus more research evidence including wider geographic coverage of Nigeria is still required.

Keywords: Diversity, Haplotype, Muscovy duck, Nucleotide, Polymorphism.

INTRODUCTION

Intensive system of livestock production is a source of high-quality protein for the fast-growing human population and for providing additional income to poorer, small farmers. From a survey, Oteku *et al.* (2006) reported that about 64.6% of the population agreed that duck meat is recommendable as one of the ways to curb the problem of animal protein shortage in Nigeria, thus continuous breeding of ducks needs basic information on their genetic background and diversity. Development of a global database containing detailed information on the genetic diversity of farm animals may prove a useful tool in the conservation and restoration of endangered species in the future (Semik and Krawczyk, 2011).

Genetic divergence studies may be used to evaluate the behaviour of genotype in different environment and also to

evaluate the superiority of some genotype over others. Gradual erosion of the genetic integrity of the existing local stock, through crossbreeding and upgrading programmes, is a cause for concern and there is need to be more focus and work against the process that is leading to erosion of our local and indigenous livestock genetic resources through maintenance of their genetic relationships between populations. This can also be done by identification of the differences in the allele number and frequency of polymorphic loci at molecular level, couple with understanding of the history of our livestock species and their evolutionary processes. Differences in the extent of polymorphism at different regions of the genome may reflect different evolutionary histories. In ducks, genetic diversity studies of some populations using random amplification polymorphic DNA (RAPD) and amplification fragment length polymorphism (AFLP) have been reported (Chen *et al.* 2001, Zuo *et al.* 2004, Yan *et al.* 2005).

Muscovy ducks are regarded as valuable birds that attract higher prices than other indigenous poultry species in different live bird market across Nigeria (Sola Ojo *et al.*, 2020). Studying their genetic diversity and relationships will provide useful information for a planned breeding programme as ducks owners practiced random mating in Nigeria. This would also be useful in designing future breeding programme for continuous production and hybrid vigour.

Therefore, this study was designed to estimate the genetic diversity of Muscovy duck from five different locations in Baruten local government, Kwara state, Nigeria using the nuclear CYP2U1 genes.

MATERIALS AND METHODS

Animal Sampling

A total of 40 adult Muscovy ducks were sampled from four different locations in Baruten local government area of Kwara state, Nigeria. Namely: *Bani* (BON), *Yenbeleku* (BART), *Ilesha Baruba* (ILE), *Okuta* (OKE). About 2ml of fresh blood was collected from the Muscovy ducks through wing venipuncture using a 2ml syringes and needle; cross contamination was totally avoided. The blood samples were preserved in 95% ethanol and transported to Kunming Institute of Zoology, Chinese Academy of Sciences, China for genomic DNA extraction, polymerase chain reaction (PCR) and genomic sequencing of the nuclear CYP2U1 gene.

Polymerase Chain Reaction and DNA Sequencing

The 747 base pair nuclear DNA CYP2UI gene fragment was amplified using forward and reverse primers P103 (5-GTTATTTGG TTAGCATATCGTG -3) and (5-GAGACGGTTGCGTATATGG -3). PCR amplification was carried out on a thermocycler with a 25ml volume reaction mixture, which include 50ng of genomic DNA, 12.5µl of master mix (Promega, Fitchburg, WI) and 2µl of (10pmol) of each primer. The PCR was performed at 950C for 5 min, followed by 30 cycles at 940C for 30 s, 1 min annealing at 450C, 1 min at 720C and a final extension at 720C for 5 min. The amplified PCR products were sequenced using the Big Dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) on ABI3500 Genetic Analyzer.

Data Analysis

The CYP2U1 gene sequence was manually edited aligned and an unrooted neighbor-joining (NJ) typology was drawn using Clustal W implemented in MEGA 7.0.18 (Tamura *et al.* 2013) and using the Kimura two parameter model of nucleotide (Kimura, 1980). All samples were analyzed for genetic diversity

indices such as number of haplotypes, haplotype diversity, polymorphic sites and number of mutations using DNASP. 6.12.03 version, (Rozas 2009). The sequences were further subjected to Multiple Sequence Alignment viewer (Jalview 2.11.7.4) using Clustal × 2.0 (Larkin *et al* 2007) to view the sequence alignment. The number of sequence was counted using G~C genomic calculator, while the accession number were obtained after submission of the sequence to NCBI.

RESULTS AND DISCUSSION

Table 1 showed the sample locations and their codes, coordinates of the sampling sites, number of sequence obtained per location and their Accession number. A total of 21 sequences were obtained from the 40 Muscovy ducks sampled in four (4) locations of Baruten Local government, Kwara State, Nigeria (6 each from *Yembeleku* and *Bani;* 7 and 2 sequences from *Ilesha Baruba* and *Okuta*, respectively). The map and coordinates of the sampling sites is as shown in Figure1. Molecular genetic characteristics and diversity among Muscovy duck found in selected locations of Baruten local government area in Kwara state was provided in this study, with twenty-one sequences obtained from the 40 Muscovy sampled in the studied area; Okuta Muscovy duck had only two sequences this might be as a results of poor quality DNA extracted from blood of the Muscovy duck from Okuta.

Table 1: Study Locations Identification, Sequences obtained and their Accession Number.

Sample Locations	Location ID	Coordinates of the Sampling Sites	Number of sequences obtained	Sequence Code	Accession Number
YEMBELEKU	BART	9.3493^0N; 3.5813^0E	6	BAT293	MZ383452
				BART394	MZ383453
				BART495	MZ383454
				BART596	MZ383455
				BART697	MZ383456
				BART899	MZ383457
BANI	BON	9.6062^0N; 3.9419^0E	6	BON3	MZ383462
				BON7	MZ383463
				BON9	MZ383464
				BON10	MZ383465
				BON20	MZ383466
				BONI25	MZ383467
ILESHA BARUBA	ILE	$8^0$55'0"N; $3^0$25'0"E	7	ILE1	MZ383470
				ILE6	MZ383471
				ILE12	MZ383472
				ILE16	MZ383473
				ILE23	MZ383474
				ILE31	MZ383475
				ILE32	MZ383476
OKUTA	OKE	$9^0$13'0"N; $3^0$10'59"E	2	OKE8	MZ383502
				OKE34	MZ383503

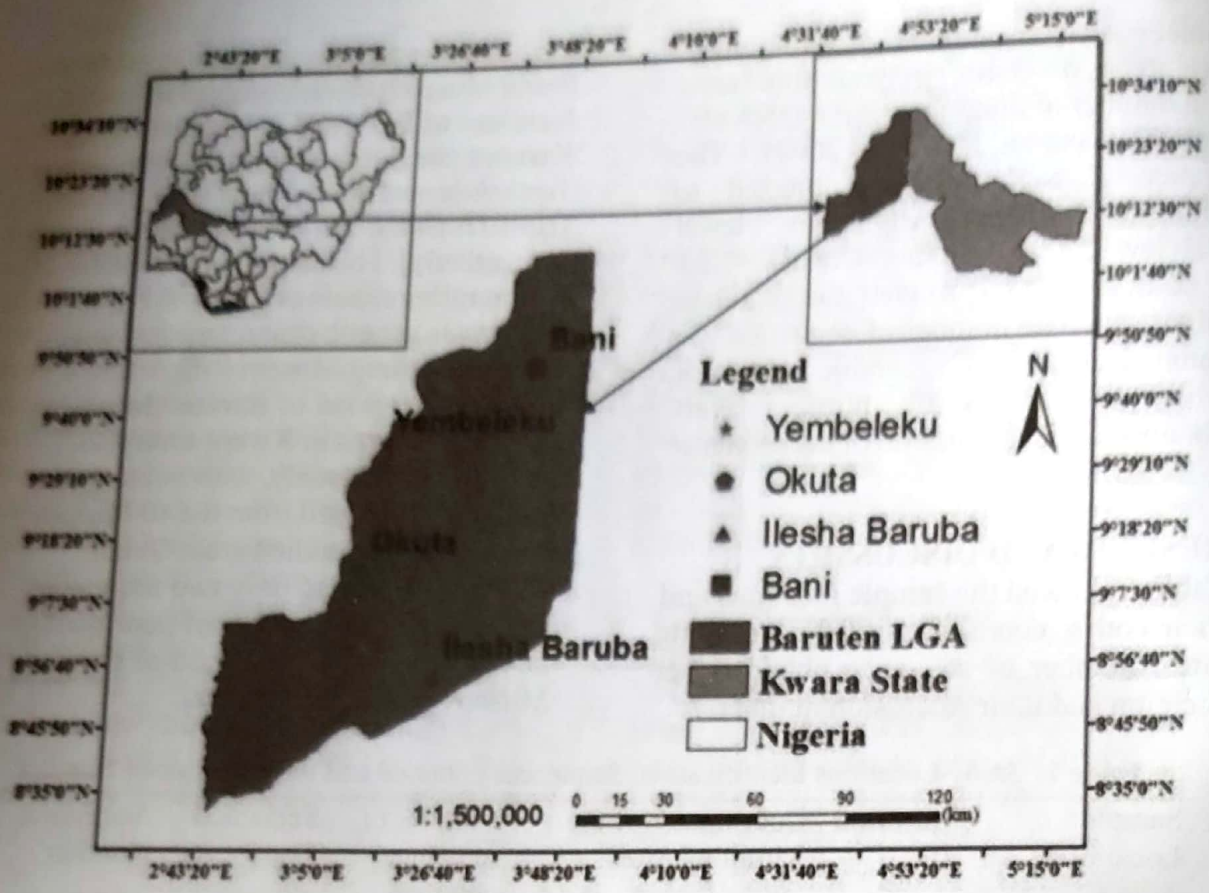


Figure 1: Map of the Sampling locations and their coordinates.

The results of the haplotype diversity and amino acid bases of the total sequences obtained are presented in Table 2. The total length of the sequence studied was 747 base pair with total of 25 polymorphic sites or approximately 6.45 of the full length, the polymorphic site consists of substitutions with 16 singletons variables comprising of single variables of two variants and three variants beam in the order of 15 and 1, respectively. The nucleotide diversity per site was 0.00608; the total numbers of mutations were 26 while the parsimony informative sites were 9. The total numbers of bases obtained were 15,697 of 33.01%, 31.22%, 17.17% and 18.59% A, T, G and C, respectively. The percentage A ~ T combined was 64.24% which is uniquely higher than the % G~ C content of 35.76%.

Table 3 showed that the haplotype diversity in BART, ILE and BON ranged from 0.80 to 0.87, it was highest in Yembeleku Muscovy duck followed by those from in Ilesha- Baruba and Bani with haplotype diversity of 0.857 and 0.800, respectively; no haplotype diversity was recorded in the two sequences obtained for Okuta. The overall haplotype diversity of all the sequence varied from 0 to 0.867, with haplotype number of 1to 5, and polymorphic sites of 0 to 20. The variance of haplotype diversity ranges from 0 to 0.029 while the nucleotide diversity ranges from 0.00 to 0.00608. Muscovy duck from Bani had the highest polymorphic sites (20), while those from Okuta have no polymorphism. The overall percentage of the haplotypes diversity in the study was 77.1 and this ranged from 80 to 87% among the 4

locations studied (Muscovy duck of BART, BON and ILE had high haplotype diversity) with low nucleotide diversity of 0.00608 and this indicates a high number of closely related haplotypes as suggested by Mendez-Harclerode *et al.* (2009) and Sola-Ojo *et al.* (2021) and not deviated from the fact that higher contribution of multiple maternal lineage occurred across the location as asserted from the findings of Liu *et al.* (2004); Hoque *et al.* (2013) and Liao *et al.* (2016) where maternal lineage sharing were reported among different indigenous chickens of various geographical locations, and also that of Tixier-Biochard *et al.* (2008) where the author reported that high genetic diversity is common among poultry populations observed in villages' populations especially in those that are separated by wide geographical distances, high haplotype diversity obtained here might be due to high level of genetic intermixing within the Muscovy duck populations in the four locations of study.

Table 2: Haplotype and Nucleotide diversity, Multi Domain Analysis results, and Amino acid bases of the total Sequences obtained.

S/N	Parameters	Values
1	Total Number of sequence	21
2	Site with alignment gaps	0
3	Invariable (Monomorphic Sites)	722
4	Variable (Polymorphic Sites)	25
5	Nucleotide diversity per site	0.00608
6	Haplotype diversity	0.771
7	NHaplotype	9
8	Total Number of Mutations	26
9	Singleton Variable sites	16
10	Singleton Variable sites (two variants)	15
11	Singleton Variable sites (three variants)	1
12	Parasimony informative sites	9
13	AminoAcids (Genomic calculations)	
13.1	Total Bases	15697
13.2	Adenine (A)	5183
13.3	Thymine (T)	4901
13.4	Guanine (G)	2695
13.5	Cytosine (C)	2918
13.6	% G~C	64.24%
13.7	% A~T	35.76%

Table 3: Haplotype and Nucleotide diversity from Sequences of Muscovy ducks Sampled

SEQ_ID	NSEQ	NHAP	PS	V/HAP	HD	P_I
BART	6	4	4	0.0167	0.867±0129	0.00205
ILE	7	5	12	0.0188	0.857±0.137	0.00688
BON	6	4	20	0.0296	0.800±0.172	0.01080
OKE	2	1	0	0.0000	0.000±0.000	0.00000

SEQ ID: - Muscovy duck identification; NSEQ: Number of sequence; NHap: Haplotype number; PS: Polymorphic Sites; V/Hap: Variance of haplotype diversi ty; HD: Haplotype diversity; P_i: Nucleotide diversity.

The phylogenetic tree (Figure 2) shows that most of the sampled Muscovy ducks from the four locations clustered into two clades showing that two major haplogroups were identified for the Muscovy ducks studied in Baruten local government area of Kwara state, Nigeria. The results also shows that there is a close monophyletic relationship between Muscovy ducks from Bani and Ilesha Baruba as seen in the upper polyphyletic clade of the phylogenetic tree (from ILE1 to BON 20), under this clade BON3 vs. ILE 3; BONI25 vs. ILE 16 and BAT293 vs. BART697 are monophyletically cladded. Within the polyphyletic clade relating OKE 8 and ILE1, BART 899 and BON7; BART 495 and 394; BON 10 and ILE6; ILE32 and OKE8 are monophyletically related, other Muscovy ducks sampled are polyphyletically cladded.

The neighbour-joining phylogram revealed a clustered relationship where some were monophyletically cladded with the exception to BON 20 which was of far distance from other Muscovy duck, and this supported the presence of many closely related haplotypes and in line with the reports of Mendez- Harclerode *et al.* (2005) and this could not be far from the fact some haplotypes clades have wider distribution across the location that might be due to human activities such as sale of farm animal, gifting of animals to visiting family members and friends, as well as their use in traditional and ceremonial rites, all these can influence the migration of Muscovy duck across the locations and results in constant flow of gene across the populations.

Figure 3 shows Jalview of the aligned sequences with respect to the consensus sequence, and some polymorphism from 0 to 167bp of the sequence. At 8 bp, there were substitution of T for C compared to the consensus and these substitutions occurred in BON 31, BONI25, ILE 16, 23 and 31. At bp 33 and 37 insertion of T for C occurred in BART 899 and ILE 23, while insertion of C for G occurred in BART 899 at 47 bp.

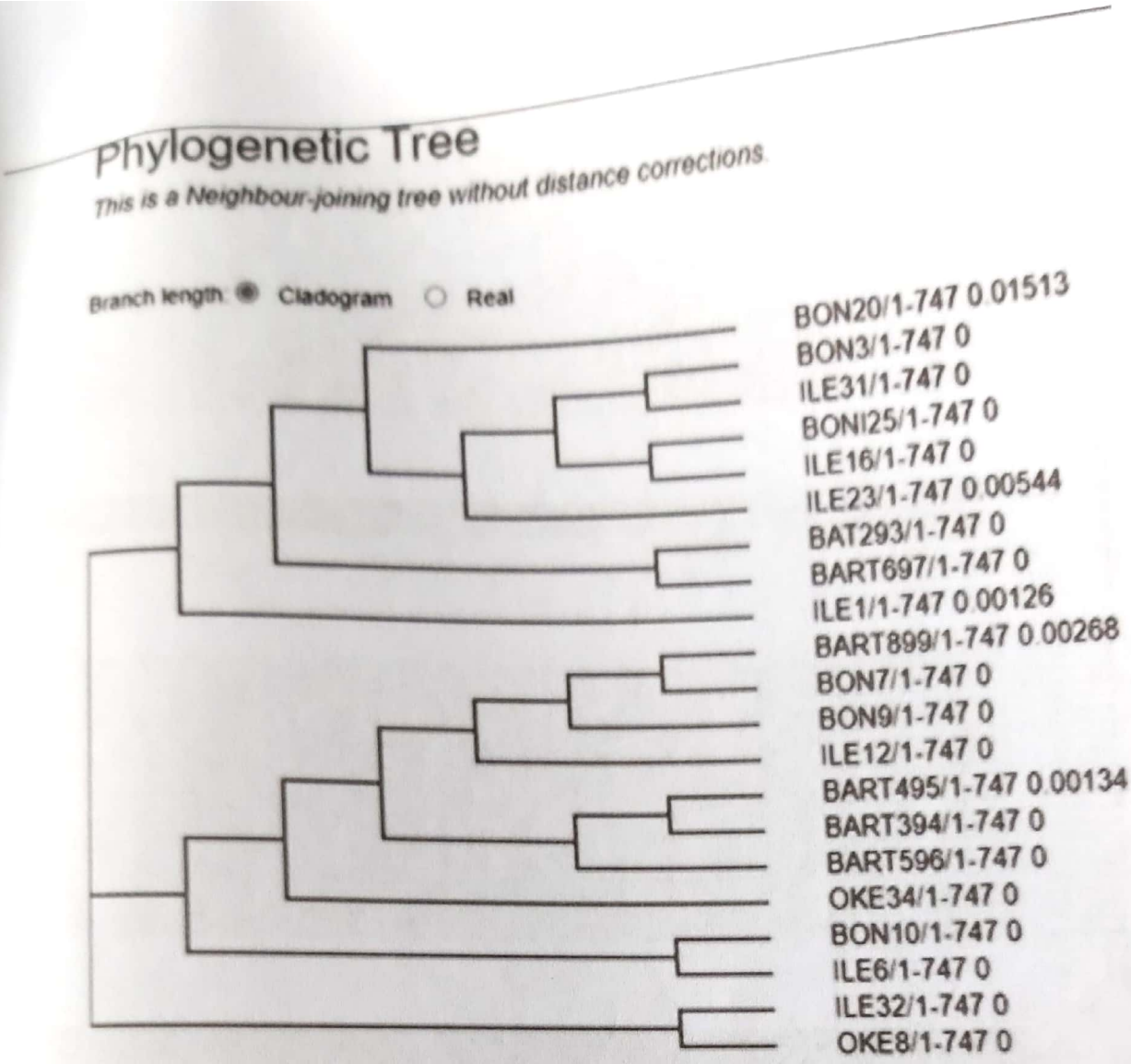


Figure : Genealogical relationship among Muscovy duck samples using the Neighbour-Joining method.

This result also shows substitution of C for T at 113bp in BON 3 and BONI 25; ILE 16 and 31. At 159 bp, there were substitutions of A for G in BON3, and 35; ILE 16, 23 and 31. The two sequences obtained for OKE had no polymorphism. The polymorphism seen in the Jalview consist of substitution and insertion in some of the Muscovy duck studied, this shows some level of variations in the population studied and could be said that they are genetically diverse and will have a positive response to selection.

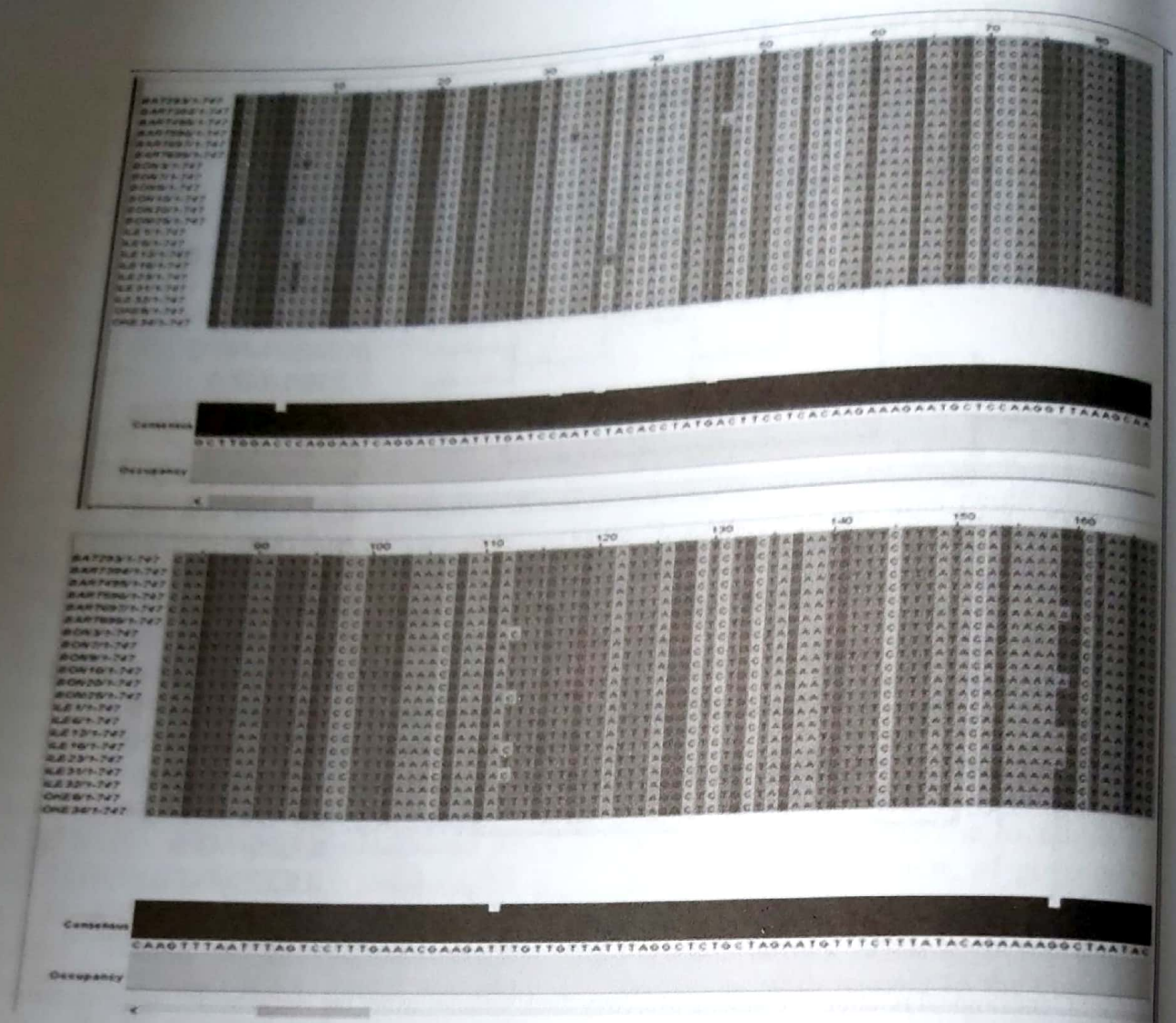


Figure 3. The Jalview of the 21 sequences showing some polymorphism at 33, 37, 47, 113 and 167 bp.

CONCLUSION AND RECOMMENDATION

Our findings shows that nuclear CYP2U1 revealed a high haplotype diversity but a low nucleotide diversity within and between the Muscovy duck studied in Baruten local government of Kwara State, Nigeria. The high haplotype diversity is in accordance with the high number of haplotypes observed in each location possibly due to sales and gifting of Muscovy duck among duck lovers as well as families and the low nucleotide diversity reflects that Nigeria Muscovy duck are likely a descent of common maternal lineage. This finding serves as a preliminary information and further research evidence from genetics and archaeology including wider geographic coverage of Nigeria is still required.

REFERENCES

Chen, Y. X. and Zuo, Z. H. (2001). Study on genetic relationship and diversity in ducks by RAPD. *Journal of Xiamen University (Natural Science),* 40(1):141-145.

Hoque, M.R., Seo, D.W. and Jo, C. (2013). Reconstruction of phylogenetic relationships of Korean chickens using

mitochondrial D-loop sequences. *Journal- Faculty of Agriculture, Kyushu University*, 58(2): 287–293.

Larkin, M.A., Blackshields, G., Brown, N., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F.,Wallace, I.M.,Wilm, A., Lopez, R. (2007). ClustalW and ClustalX version 2.0. *Bioinformatics*, 23 (21): 2947–2948.

Liao, Y., Mo, G., Sun, J. Wei, F., Liao, D.J. (2016). Genetic diversity of Guangxi chicken breedes assessed with microsatellites and the mitochondria DNA D-loop region. Molecular Biology reports 43: 415-425.

Liu, G. Q., X. P. Jiang, J. Y. Wang and Z. Y. Wang. (2006). Correlations between heterozygosity at microsatellite loci, mean d2 and body weight in a Chinese native chicken. Asian-Aust. J. Anim. Sci. 19(12):1671-1677.-50.

Mendez-Harclerode F.M., Hanso, J.D. Fulhost, C.F., Milazzo, M.L. Ruthven, DC., III, Bradley, R.D. (2005). Genetic diversity within the southern plains woodrat (Neotoma micropus_in Southern plains woodrat (Neotoma micropus) in South Texas, Journal of Mammalogy. 2005; 85:180-190.

Mendez- Harclerode, F.M., Strassus, R.R., Fulhost, C.F., Milazzo, M.L. Ruthven D.C., III and Bradley R.D. (2009). Journal of Mammalogy, 88(2):360-370.

Oteku, I.T., Igene, J.U. and Yessuf, I.M. (2006) An assessment of the factors influencing the consumption of duck meat in Southern Nigeria. Pakistan Journal of Nutrition 5: 474-477.

Rozas A, Ladoukakis, E, Zouros, E (2009) Animal mitochondrial DNA recombination revisited. Trends in Ecology and Evolution, 18:411–417.

Semik E. and Krawczyk, J. (2011) The state of poultry genetic resources and genetic diversity of hen populations. Annals of Animal Science 11: 181-191.

Sola-Ojo, F.E, Adeola, AC, Yusuf, O.A, Abiola, J. (2020) Phenotypic, Physiological and Blood Profile Characterization of Muscovy Ducks (*Cairina moschata*) In North Central Nigeria. Genetics and Biodiversity Journal, 4 (1): 30–39.4.

Sola-Ojo, F.E., Adeola. C.A. Lamarck A.O. Yusuf, A.O. Momoh, E.O, Adekoya, A.R and Adedibu, I.I. (2021). Genetic diversity and relationship of Nigerian Muscovy duck populations. Journal of Animal Breeding and Genomics 5(1): 1-9.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution, 30(12): 2725–2729.

Tixier-Boichard, M., Bordas, A. & Rognon, X. 2008. Characterization and monitoring of poultry genetic resources. Proceedings of the 23rd World' s Poultry Congress, Brisbane, Australia, July 2008. CD-ROM.

Yan, F. H., ZH. H. Zuo, M. Chen, Y. Q. Song, L. J. Lu and Y. X. Chen. 2005. Analysis of genetic diversity and relationship among some Chinese domestic ducks and wild ducks using AFLP. Journal of Xiamen University (Natural Science). 44(5): 729-733.

Zuo, Z. H., Y. X. Chen and L. J. Lu. 2004. Studies on genetic diversity and molecular marker of Jinding duck. Journal of Xiamen Universit (Natural Science) 43(2):256-259.