Nigerian J. Anim, Sci. 2024, 26 (2): 30-45

Genetic Characterization of Southern Nigerian Indigenous Turkey Using Mitochondrial DNA

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Target Audience: Animal Geneticists, Biotechnologists, Researchers and Students

Abstract

The first Hypervariable (HV1) region of the mtDNA of 84 Nigerian indigenous turkey (NIT) sampled from Rivers, Kwara, Ebonyi, Oyo and Lagos states were amplified using DBF; 5'-AGGAGGAGGAGACCCAATCC-3 and 5'-CAGTGGGTGAAGCCTGCTAA-3' as forward and reverse primers respectively. PCR amplicons were sequenced using the Sanger sequencing method. The sequences were viewed using Bioedit software, edited, trimmed, aligned and single nucleotide polymorphism was identified using MEGA 11. Diversity indices were estimated using ARLEQUIN software. The genetic distances and phylogenetic relationship between the mtDNA sequences of the NIT and those of 12 birds obtained from Genbank were estimated using the pairwise distance and Neighbor-joining method of MEGA 11 respectively. Also, an analysis of molecular variance was used to determine the population structure using ARLEQUIN software and the demographic indices were also estimated using DnaSP v6.

Result from this study showed a non-synonymous single nucleotide polymorphism at position g.6170(A>G) in eighteen samples and two haplotypes (Hap_1 and Hap_2) were detected. The entire population showed low haplotype diversity (0.3409) and very low nucleotide diversity (0.00073). The genetic distances estimated between sampled populations were low (0.00), which showed they were very related. The phylogenetic tree further confirmed the degree of closeness observed in the estimated genetic distance values. Analysis of molecular variance showed that the genetic variation among populations (8.64%) was lower than the genetic variation within populations (91.36%). This observation and the low fixation index indicated that the sub-populations were not differentiated. The demographic indices showed that some sub-populations were evolving neutrally while some were expanding.

Keyword: Nigerian indigenous turkey; mtDNA; Genetic characterization; Single nucleotide polymorphism.

Description of problem

Mitochondrial DNA (mtDNA) is a tiny DNA, circular in shape, usually found in eukaryotic cells' mitochondria [1] which has been used for the characterization of several livestock species [2,3,4,5,6]. It is smaller than the nuclear genome in size and encodes 37 genes, 24 of which code for tRNA and rRNA, and 13 of which are required for the respiratory chain to function [7]. In comparison to nuclear DNA, mtDNA is far more polymorphic, evolving at a rate five to ten times quicker than that of the nuclear genome. Because various mtDNA areas change at different rates, it is a preferred marker for researching genetic diversity both within and between species. Compared to other mtDNA gene sections, the displacement (D)-loop region changes considerably more quickly and is non-coding. According to [8], this makes it especially helpful for phylogeographic study. In most species, mtDNA is inherited maternally and does not undergo recombination [9]. Because of these characteristics, every molecule has typically only one maternal lineage-based genealogical history.

The Nigerian indigenous turkeys (NIT) are hardy, better adapted to tropical environmental conditions compared to exotic turkeys such as Hybrid converter, British United and Nicholas White turkeys. They are cheaper to raise, natural foragers, range farther and have better meat quality [10]. The skin and plumage colours of these birds are just two of the many phenotypic variations they exhibit [11]. In NIT, there are three different colours of plumage: black, lavender/bronze, and white [12]. Being among the biggest species of poultry, the turkey weighs between 10-15.8 kg live weight [13]. Turkey is simple to keep as domestic animals, with a relatively high profit and quick returns on investment, according to [14]. Indigenous livestock breeds are significant due to both their traditional husbandry techniques and tolerance to local biotic and abiotic challenges.

The knowledge of genetic diversity is important as it forms the basis for designing breeding programs and making rational decisions on sustainable utilization of animal genetic resources [15], and selection. Nigeria has a remarkable diversity of indigenous livestock, the potential of which should not be underestimated given the massive foreign exchange implications of importing enhanced exotic stock, as well as the genotype-environment interaction that has resulted in significant loss of fitness of the exotic stock. Despite the economic and genetic advantages of the Nigerian indigenous turkey, there is no available information on characterization using molecular markers such as mitochondrial DNA. Therefore, the objective of this study is to characterize the Southern-Nigerian indigenous turkey populations using the first hypervariable (HV1) region of mitochondrial DNA (mtDNA) marker.

Materials and Methods

Sampling locations

This study was carried out using blood samples collected from Indigenous turkeys in Lagos State located at 6°27'14.65"N, 3°23'40.81"coordinates, Oyo State located at 8.1574° N, 3.6147° E coordinates, Kwara State located at 8.9669° N, 4.3874° E coordinates, Rivers State located at 4.8396° N, 6.9112° E coordinates and Ebonyi State

located at 6.2649° N, 8.0137° E coordinates in Nigeria.

Experimental animals

A total of 84 indigenous turkey birds (Ebonyi =13, Rivers =8, Kwara = 23, Oyo=31, Lagos = 9) were used for this study. These animals were sampled from various farms and households where they were being managed intensively or semi-intensively.

Blood sample collection

Using needles and syringes, 0.5ml of blood samples were drawn from the turkeys' wing vein by licensed veterinarians and dropped onto the Fast Technology for Analysis (FTA) cards (Whatman Bioscience, UK). Extreme temperature was avoided, and the samples were kept in sealed plastic bags after they had been allowed to dry at room temperature. Blood samples were taken to the lab for DNA extraction.

Genomic DNA extraction

DNA was extracted from air-dried blood spotted on FTA classic cards at the Biotechnology lab of ACUTIG Nigeria Limited, Abeokuta, Ogun state.

DNA Quantification

DNA quantification was performed to determine the concentration of the DNA samples prior PCR using the NanoDrop spectrophotometer. Precautions were taken to avoid contamination of the DNA samples.

Primers and PCR amplification

A pair of primers was designed and used to amplify the 468bps of first hypervariable (HV1) region of the mtDNA based on the reference sequence from the Genbank (EF153719) by African Biosciences Limited (Nigeria).

F; 5' -AGGAGGAGGAGACCCAATCC-3' (20bp),

R; 5' -CAGTGGGTGAAGCCTGCTAA-3' (20bp).

For amplification, a 20µl reaction mixture was prepared. This mixture consisted of 2µl genomic DNA, 0.4 µl of primers (forward and reverse), 13.2µl double distilled water and 4µl FIREpol master mix (solis biodyne), containing 7.5mM $MgCl_2$, 1mM dNTPs, blue dye, yellow dye and 5x reaction buffer containing 0.4M Tris-HCL. The PCR conditions included 35 cycles initial denaturation at 95^{O}C for 30 seconds, final denaturation at 95^{O}C for 30 seconds, annealing at 55^{O}C for 30 seconds, extension at 72^{O}C for 40 seconds, and final extension at 72^{O}C for 3 minutes.

Gel electrophoresis

Plates 1, 2, 3 and 4 show the PCR products of the HV1 region of mtDNA as revealed by gel electrophoresis for the samples. Two percent (2%) agarose gel was used for the electrophoresis. 0.8g of agarose powder (CSL-AG100 LE multi-purpose agarose) was dissolved in 40 ml of 1X TAE (TRIS acetate EDTA) electrophoresis buffer using a microwave to ensure the powder dissolved properly. The solution was then stained with 9µl ethidium bromide and was allowed to polymerize in the gel electrophoretic cast in which the comb was properly placed. TAE running buffer was poured into the electrophoresis tank to submerge the polymerized gel. The amplified PCR product of each sample was resolved in the 2% agarose gel at 80v, 250mA for 15 minutes. 2µl of the products were carefully

loaded in the wells, using 100bp molecular weight marker as the control for size. Resolved allelic fragments were sized using 100bp ladder and were visualized using UV transilluminator.

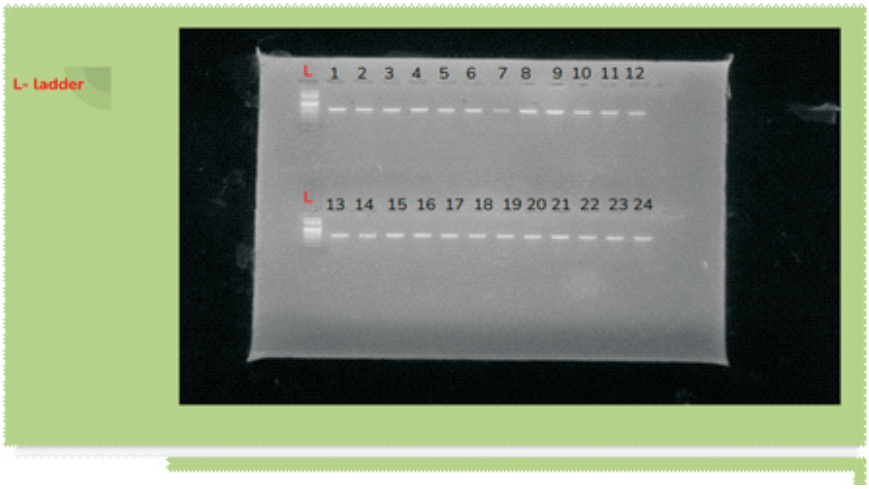

Plate 1: Electropherogram of the HV1 region of mtDNA as revealed by gel electrophoresis for samples 1-24.

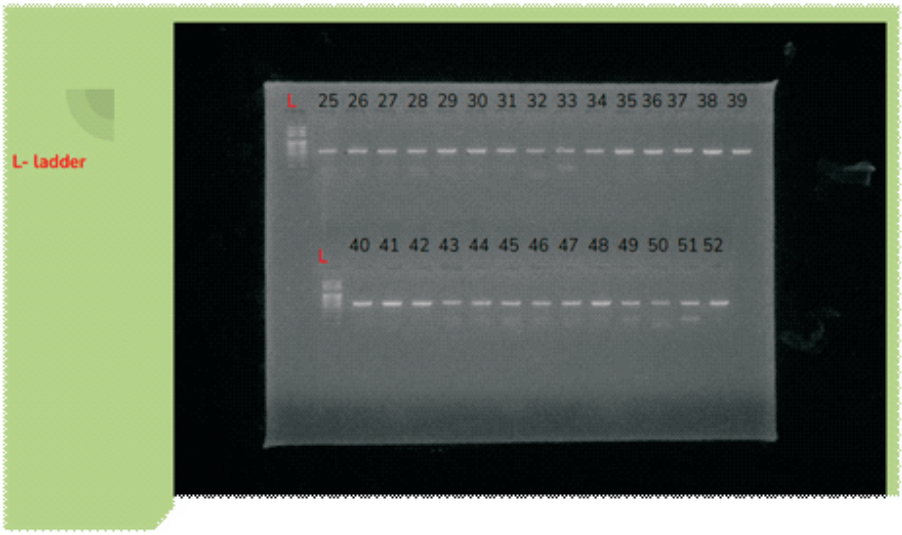

Plate 2: Electropherogram of the HV1 region of mtDNA as revealed by gel electrophoresis for samples 25-52.

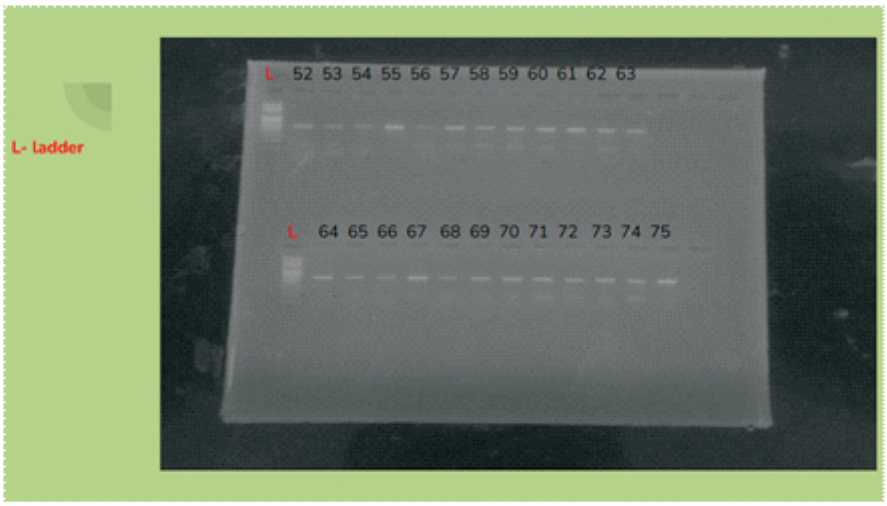

Plate 3: Electropherogram of the HV1 region of mtDNA as revealed by gel electrophoresis for samples 52-75.

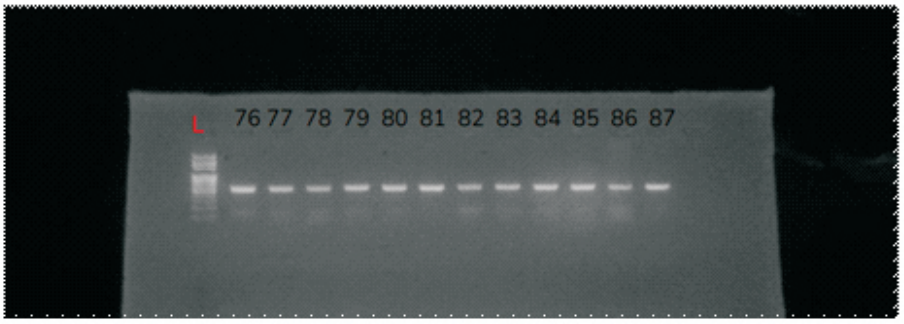

Plate 4: Electropherogram of the HV1 region of mtDNA as revealed by gel electrophoresis for samples 76-84.

DNA sequencing, viewing and alignment

The sequencing was performed using Applied Biosystems 3730x1 DNA analyzer at the African Biosciences Limited, Nigeria. The sequences were viewed with Bioedit software [16]. Molecular Evolutionary Genetics Analysis (MEGA) version 11 was used for the alignment of sequences and identification of single nucleotide polymorphisms.

Nucleotide and haplotype diversity

Nucleotide and haplotype diversities were estimated with regions shared by the DNA sequences of the birds using the ARLEQUIN software [17]. Also, haplotype frequency and other molecular diversity indices were estimated.

Genetic distance

A consensus sequence was generated for each of the Southern-Nigerian indigenous turkey populations using the BioEdit software [16]. In addition to these sequences, 12 sequences obtained from the NCBI GenBank database (Table 1) were also estimated using the pairwise distance method of the MEGA 11 software [18].

Table 1: Geographic and identity details of sequences obtained from Genbank

Bird	Source	Accession number	References
Blue slate turkey	USA	EF153719	19
Nicholas turkey	USA	NC010195	20
Ocellated turkey	Mexico	KU094576	21
Indigenous turkey	Egypt	MK284430	22
Indigenous turkey	Iran	MK284450	22
Indigenous turkey	Brazil	MK284411	22
Muscovy duck	Nigeria	MN942261	23
Muscovy duck	China	MN942262	23
Muscovy duck	India	GQ922096	24
Indigenous chicken (Fulani ecotype)	Nigeria	MN010436	25
Indigenous chicken (Yoruba ecotype)	Nigeria	MN010490	25
Indigenous chicken	Japan	AB007720	26

Evolutionary relationship, population structure and demographic history

Phylogenetic trees were constructed using the Neighbor-joining method on MEGA 11 software [18]. An Analysis of Molecular Variance (AMOVA) was used to calculate the genetic variation within and among populations. In addition, F_{ST} values from pairwise comparisons were also computed with 1000 permutations using ARLEQUIN V3.1 [17]. The Tajima's D [27] and Fu's Fs [28] values of the sampled Nigerian indigenous turkey were estimated using DnaSP v6.

Results and Discussion

Identification of single nucleotide polymorphism (SNP) and amino acid variation

The amino acid variation and effect of SNP identified in the HV1 region of mtDNA of the Southern-Nigerian indigenous turkey population is presented in Table 2. Sequencing and analyses of the first hypervariable (HV1) region of mtDNA showed a single nucleotide polymorphism at position g.6170(A>G) in 18 (Kwara=2, Lagos=1, Ebonyi=5, Oyo=10) out of 84 samples. The presence of the SNP in specific populations (Kwara, Lagos, Ebonyi, Oyo) suggests a certain degree of genetic diversity within and between these populations. The uneven distribution (e.g., more prevalent in Oyo) may indicate historical population dynamics, such as migration, genetic drift, or selective pressures.

The SNP found is Transition i.e. the mutation changed from a purine nucleotide to another purine nucleotide. The mutation is non-synonymous which is predicted to cause a

change in the amino acid produced. According to [29], non-synonymous mutations lead to amino acids change and it changes the protein structure and functions. The transition which is also non-synonymous can contribute to genetic stability by maximizing the impact of protein function [30]. Also, the predicted change in protein function can provide a selective advantage in certain environments and can lead to effects on phenotypic traits and development [31].

The number of variations identified in this study is lower than the variation reported by [5] who analyzed relatedness among turkey and reported 18 variations with the use of mtDNA marker. [22] also traced worldwide turkey genetic diversity by using the d-loop mtDNA and reported 76 variations. The low variation reported could be due to the homogenous nature of the populations studied, possibly attributed to few founders and perhaps high level of inbreeding in the Southern-Nigerian indigenous turkey populations.

Table 2: SNP identification and amino acid variation based on the HV1 of the mtDNA in turkey populations

Sample id	Polymorphism	Codon change	Amino acid change	Effect of change	Mutation type
LA5L, KW22B, KW25L, EB14B, EB18W, EB20W, EB21W, EB2W, OY51L, OY12B, OY16B, OY19W, OY31B, OY32L, OY4B, OY38W, OY3W, OY49L	g.6170 A> G	AGG> GGG	Arginine> Glycine	Non-synonymous	Transition

LA=Lagos, KW=Kwara, EB=Ebonyi, OY=Oyo

Nucleotide and haplotype diversities of the HV1 region of mtDNA in the sampled NIT populations

The diversity indices were estimated for the sampled NIT populations and two turkey haplotypes were detected (Table 3). The dominant haplotype was Hap_1 (n=66) and it was present in 78.57% of the total population, sharing a maternal line with 8 Rivers individuals, 8 Lagos individuals, 8 Ebonyi individuals, 21 Oyo individuals and 21 Kwara individuals. The total population analyzed (n=84) showed low haplotype diversity (0.3409) and very low nucleotide diversity (0.00073). Table 4 shows the values of some molecular diversity indices including the nucleotide and haplotype diversities of the populations. Also, Table 5 shows the Haplotype frequencies of the sampled NIT populations. Considering different sub-populations, the haplotype diversities ranged from 0.0000 to 0.51282 and nucleotide diversities ranged from 0.0000 to 0.0011. The Ebonyi turkey population had the highest diversity indices while the Rivers turkey population had the lowest.

Haplotype diversity reflects allelic differences among samples, while nucleotide diversity indicates the average number of nucleotide differences among DNA sequences [32]. The diversity indices obtained in this study were generally low and could be attributed to the low level of allelic variation detected in the sequences used for the study which shows that they are closely related. It could also be due to population founder effect or bottleneck effects. This result is in contrast with [5] who reported 15 haplotypes using 126 birds from 15 different turkey varieties, haplotype diversities ranged from 0.00 to 0.83, nucleotide diversities also ranged from 0.00 to 0.08 and relative frequencies ranged from 0.009 to 0.333 in their study. This might be due to the different varieties of turkey used in their study. Also, [22] reported 3 haplotypes with haplotype diversity of the total population estimated at 0.596 and the nucleotide diversity was 0.004. The low Hd and π values estimated in this study were similar to estimates by [25] for Nigerian indigenous chickens using mtDNA.

Table 3: Haplotype frequency of the total NIT population based on HV1 of mtDNA

Haplotype	N	Haplotype frequency
Hap_1	66	0.79
Hap_2	18	0.21

n: Sample size

Table 4: Some Molecular diversity indices based on HV1 of mtDNA estimated for the sampled NIT populations

Indices	Kwara	Ebonyi	Lagos	Rivers	Oyo
n	23	13	9	8	31
H	2	2	2	1	2
Hd	0.1660±0.0976	0.5128±0.0822	0.2222±0.1662	0.0000±0.0000	0.4516±0.0629
π	0.0004±0.0005	0.0011±0.0011	0.0005±0.0007	0.0000±0.0000	0.0010±0.0010
N	1	1	1	0	1
K	0.1660±0.2322	0.5128±0.4612	0.2222±0.2880	0.0000±0.0000	0.4516±0.4198
C%	26.71	26.71	26.71	26.71	26.71
T%	28.42	28.42	28.42	28.42	28.42
A%	26.26	26.20	26.26	26.28	26.21
G%	18.61	18.67	18.61	18.59	18.66

n: Sample size, h: number of haplotypes, Hd: haplotype diversity, π: Nucleotide diversity, N: number of polymorphic sites, k: mean number of pairwise differences, C%: Cytosine, T%: Thymine, A%: Adenine, G%: Guanine.

Table 5: Haplotype frequencies of the sampled NIT populations

Haplotype	Rivers	Lagos	Ebonyi	Kwara	Oyo
Hap_1	8	8	8	21	21
Hap_2	0	1	5	2	10

Genetic distances between the sampled NIT populations and other published species

The genetic distances between the sampled NIT populations and other published species are shown in Table 6. The genetic distance estimated using Pairwise distance method showed very low values within the sampled populations (0.00). The degree of closeness shows that they are very related. Also, there were very low estimated values between the Brazilian and Egyptian indigenous turkey (0.000), Yoruba and Fulani ecotype chickens (0.000), showing that they are genetically close. However, the highest value (0.793) was recorded between the Blue slate turkey and the Nigerian Muscovy duck.

These estimates imply that the Southern Nigerian local turkey populations possessed very low mtDNA genetic distance resulting from homogenous population which could be as a result of high level of inbreeding. The implication of this low maternal genetic distance estimates is that the population won't be useful in selection programs due to the homogenous nature and can be easily

wiped off (extinction). The genetic distance reported in this study is in contrast with the report of [33] that ranged from 0.037 to 0.109 using 7 protein markers and the estimates indicated differentiation among the studied population. This might be due to difference in the markers used. However, the estimates reported in this study are within the range (0.000-0.058) reported by [34] for local animal breeds.

Table 6: Estimates of genetic distances between the sampled NIT populations other published species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1																	
2	0.000																
3	0.000	0.000															
4	0.000	0.000	0.000														
5	0.000	0.000	0.000	0.000													
6	0.732	0.732	0.732	0.732	0.732												
7	0.729	0.729	0.729	0.729	0.729	0.004											
8	0.730	0.730	0.730	0.730	0.730	0.043	0.039										
9	0.729	0.729	0.729	0.729	0.729	0.002	0.002	0.042									
10	0.727	0.727	0.727	0.727	0.727	0.002	0.004	0.049	0.002								
11	0.729	0.729	0.729	0.729	0.729	0.002	0.002	0.042	0.000	0.002							
12	0.764	0.764	0.764	0.764	0.764	0.793	0.790	0.772	0.790	0.788	0.790						
13	0.741	0.741	0.741	0.741	0.741	0.758	0.760	0.759	0.760	0.750	0.760	0.588					
14	0.737	0.737	0.737	0.737	0.737	0.756	0.758	0.757	0.759	0.748	0.758	0.587	0.009				
15	0.746	0.746	0.746	0.746	0.746	0.752	0.752	0.751	0.752	0.751	0.752	0.727	0.745	0.746			
16	0.746	0.746	0.746	0.746	0.746	0.753	0.753	0.751	0.753	0.751	0.753	0.727	0.745	0.746	0.000		
17	0.748	0.748	0.748	0.748	0.748	0.750	0.750	0.744	0.750	0.748	0.750	0.727	0.752	0.752	0.022	0.022	

1: Ebonyi turkey, 2: Oyo turkey, 3: Lagos turkey, 4: Kwara turkey, 5: Rivers turkey, 6: Blue slate turkey, 7: Nicholas turkey 8: Ocellated turkey 9: Egypt turkey, 10: Iran turkey, 11: Brazil turkey, 12: Nigerian Muscovy duck, 13: Chinese Muscovy duck, 14: Indian Muscovy duck 15: Fulani ecotype chicken, 16: Yoruba ecotype chicken, 17:

Evolutionary relationship

Phylogenetic trees were constructed to show the evolutionary relationship between the sampled NIT populations and other published species (Figure 1). Based on location, the neighbor-joining phylogenetic tree revealed 4 clusters. The first cluster revealed that the Ebonyi, Oyo, Lagos, Kwara, Rivers, Blue slate, Nicholas turkey are closely related while the Ocellated turkey is distantly related to them. The second cluster revealed the Iran, Brazil and Egypt turkey. The Japan chicken, Yoruba and Fulani ecotype chicken formed the third cluster while the Nigerian, Chinese and Indian Muscovy duck formed the last cluster. The mtDNA phylogenetic analysis suggests that the sampled NIT, Blue slate and Nicholas

turkey share a more recent ancestor compared to the other turkey birds and it further explains the degree of closeness observed in the estimated genetic distance values. Also, in relation to the improvement of NIT, cross-breeding of Ebonyi turkey and Nicholas turkey should be considered as they share a wider gene pool that can help the population adapt to new challenges.

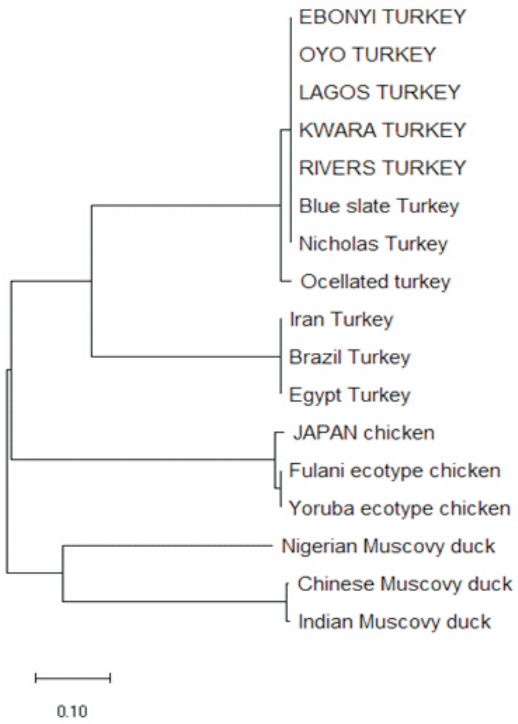

Figure 1: Phylogenetic tree showing the evolutionary relationship between the sampled NIT populations and other published species.

Population structure

An Analysis of Molecular Variance (AMOVA) was used to calculate the genetic variation within and among the sampled NIT populations (Table 10). with 1,000 permutations. Based on location, the distribution of genetic variation obtained using an AMOVA showed that the genetic variation among populations (8.64%) is lower than genetic variation within populations (91.36%). The percentage of genetic variation among populations and the low fixation index indicated that the sub populations were not well structured. Also, a low fixation index value and high variation within populations indicated a lack of genetic structure among the subpopulations.

Table 7: AMOVA showing the partition of mtDNA genetic variation within and among the sampled NIT populations according to location

Source of variation	DF	Sum of squares	Variance components	% of variation
Among populations	4	1.577	0.01504 Va	8.64
Within populations	79	12.566	0.15906 Vb	91.36
Total	83	14.143	0.17411	

Fixation Index (F_{ST}): 0.08640 P-value: 0.04790 ± 0.00641.

Demographic pattern of the sampled NIT populations

The demographic pattern for the population was examined using Tajima's D and Fu's F values (Table 8). Results showed non-significant positive (Ebonyi, Oyo) and negative (Kwara, Lagos) Tajima's D values for the populations. There was no estimated value for Rivers population because there was no polymorphism found in the population. Non-significant positive Tajima's D and Fu's F values showed that the populations are evolving neutrally while the negative values indicated very low variation which suggest that the population is expanding.

Table 8: Demographic indices of the sampled NIT populations based on Hv1 of mtDNA according to location

Population	Tajima's D	Fu's F
Ebonyi	1.30045 ns	1.151
Kwara	-0.66215 ns	-0.213
Oyo	1.24024 ns	1.459
Lagos	-1.08823 ns	-0.263

ns: Not significant P > 0.10

Conclusion and Applications

1. Low haplotype and nucleotide diversities were detected in the HV1 region of the mtDNA of the sampled NIT populations. Also, the genetic distance between the sampled populations is very close.
2. The evolutionary relationship of the HV1 region of the mtDNA revealed that the sampled NIT populations are closely related and they share a more recent ancestor with the Blue slate and Nicholas turkey.
3. The Analysis of Molecular Variance estimated in this study revealed that the sub-populations are not well

structured while the demographic indices estimated revealed that some of the sub-populations are neutrally evolving while some are expanding.

4. An association study should be carried out to investigate the effect of the non-synonymous SNP reported in the study on disease resistance and fitness for conservation of NIT population. Also, studies should be carried out on the Nigerian indigenous turkey using larger sample size to increase the chances of detecting more variations.

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