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Genetic Diversity of Mitochondrial DNA (mtDNA) *D-Loop* Sequences in Six Improved Tropically Adapted Chicken Breeds (iTABs) in Imo State, Nigeria

OKANI-ONYEJIAKA Mirian C[1*], OGUNDU Uduak E[1], BOUDALI Selma F[2], BAMIDELE Oladeji[3], OGBUEWU Ifeanyi P[1], ALADI Nnanyere O[1]

[1]*Department of Animal Science and Technology, Federal University of Technology, Owerri, Imo State, Nigeria;*

[2]*Département de Génétique Moléculaire Appliquée, Laboratoire de Génétique Moléculaire et Cellulaire (LGMC), Université des Sciences et de la Technologie d'Oran Mohamed Boudiaf USTO-MB, BP 1505, El M'naouer, Oran, Algérie ;*

[3]*African Chicken Genetic Gains, International Livestock Research Institute, Addis Ababa, Ethiopia.*

Corresponding Author: Okani-Onyejiaka MC**,** Federal University of Technology, Owerri, Imo State, Nigeria; **Email**: mirianonyejiaka@gmail.com

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Abstract

The improved tropically adapted chicken breeds (iTABs) are low-input-high-output chickens suitable for smallholder poultry (SHP). Six iTABs (Fulani, FUNAAB Alpha, Kuroiler, Noiler, Sasso and Shika Brown) were introduced, and were raised under semi-intensive management system and tested under the African Chicken Genetic Gains project in Nigeria. The objective of this study was to evaluate the genetic diversity of these iTABs tested in Imo State Nigeria using mitochondrial DNA (mtDNA), Blood samples were collected from 77 chickens belonging to these six populations of iTABs in the ratio (12:12:14:13:13:13), for Noiler, FUNAAB Alpha, Shika Brown, Kuroiler, Sasso and Fulani chickens, respectively. Genomic DNA was extracted from seventy-seven birds randomly selected from the six iTABs. A 450-bp mtDNA *D-loop* region was sequenced. The highest (H=5) and the lowest (H=2) number of haplotypes were found within Noiler, and Shika Brown/Fulani, respectively. Among the six populations, haplotype and nucleotide diversity was 0.558±0.063 and 0.0064±0.0013, respectively. A total of 8 haplotypes were identified from 15 polymorphic sites. These haplotypes clustered into three clades with 87.89% of the total maternal genetic variations occurring within population. Fulani and Shika Brown had the least (0.000) genetic distance. Tajima's D was negative among populations and within Noiler, Kuroiler, Sasso and Fulani populations but was only statistically significant within the Noiler population. Diversity indices of this study revealed that mtDNA polymorphism was on the average both within populations and among populations. The results indicate the existence of two distinct maternal lineages from Southeast Asia, south central and Southeast China evenly distributed among the iTABs. The average genetic diversity observed within population can be utilized for the long-term genetic improvement and stabilization of the breeds.

Keywords: Differentiation, iTABs, Phylogenetics, Polymorphism, Nigeria, Smallholder-poultry.

الملخص

سلالات الدجاج المحسّنة المتكيّفة استوائيًا (iTABs) هي دجاجات ذات مدخلات منخفضة ومخرجات عالية ومناسبة للدواجن ذات الحيازات الصغيرة (SHP). تم تقديم ستة iTABs (Fulani، FUNAAB Alpha، Kuroiler، Noiler، Sasso و Shika Brown)، وتم تربيتها في ظل نظام إدارة شبه مكثف واختبارها في إطار مشروع المكاسب الجينية للدجاج الأفريقي في نيجيريا. كان الهدف من هذه الدراسة هو تقييم التنوع الجيني لـ iTABs التي تم اختبارها في ولاية إيمو بنيجيريا و ذلك باستخدام الحمض النووي للميتوكوندريا (mtDNA)، تم جمع عينات الدم من 77 دجاجة تنتمي إلى هذه المجموعات الست من iTABs بنسبة (12: 12: 14: 13: 13: 13)، لدجاج Noiler، FUNAAB Alpha، Shika Brown، Kuroiler، Sasso و Fulani ، على التوالي. تم استخراج الحمض النووي الجينومي من سبعة وسبعين طائرًا تم اختيارهم عشوائيًا من ستة iTABs. تم تسلسل منطقة حلقة mtDNA *D-loop* بقدرة 450 قاعدة زوجية .تم العثور على أعلى (H = 5) وأدنى (H = 2) عدد من الأنماط الفردانية (الهبلوتيب) داخل Noiler، وShika Brown / Fulani، على التوالي. من بين المجموعات الستة، قدر تنوع النمط الفرداني والنيوكليوتيدات بـ 0.558 ± 0.063

و0.0064 ± 0.0013، على التوالي. تم تحديد ما مجموعه 8 أنماط فردانية من 15 موقعًا متغيرا. تتجمع هذه الأنماط الفردانية في ثلاث مجموعات مع حدوث 87.89 ٪ من إجمالي الاختلافات الجينية الأم داخل السكان. كان لدى Fulani وShika Brown أقل مسافة وراثية (0.000). كانت نتائج D لـ Tajima سالبة بين االعشائر وداخل مجموعات Noiler وKuroiler وSasso وFulani ولكنها كانت ذات دلالة إحصائية فقط داخل مجموعة Noiler. كشفت مؤشرات التنوع لهذه الدراسة أن تعدد أشكال mtDNA كان في المتوسط داخل العشائر وبين العشائر. تشير النتائج إلى وجود سلالتين الأم متميزتين من جنوب شرق آسيا وجنوب وسط وجنوب شرق الصين موزعين بالتساوي بين iTAB. يمكن استخدام متوسط التنوع الجيني الملاحظ داخل العشائر من أجل التحسين الجيني على المدى الطويل وتثبيت السلالات.

الكلمات المفتاحية: تمييز، iTABs ، تطور السلالات، تعدد الأشكال، نيجيريا. الدواجن الريفية.

Introduction

Smallholder poultry (SHP) production is essential for the enhancement of the socio-economic status of Nigerians and has the potential for poverty alleviation as well as wealth creation for the poorest of the poor (Sonaiya et al., 1999). However, SHP in Nigeria has little or no contribution to the country's agricultural gross domestic product (AgGDP) largely because most smallholder poultry farmers practice subsistence farming, using unimproved and low producing breeds of chicken (Sonaiya et al., 1999). Since, the major priority of today's rural poultry farmers is not only having birds that lay more eggs but also having birds that lay eggs with an optimum size as well as birds that grow to optimum body weight with plumage color similar to the indigenous birds (Mahendra, 2015), it becomes necessary to improve smallholder poultry production.

Many development projects have been put in place to improve smallholder family chicken production in Nigeria; most of which focused on control of diseases, reduction in predation and mortality through vaccination campaigns, control of internal and external parasites, and improvements in housing, feeding, management of the laying process with natural incubation and chicks' management (Wethli, 2003; Sonaiya, 2014a; 2014b). The World Bank spent many years looking for high-leverage opportunities to help national governments to invest in smallholder poultry systems using highly productive exotic genotypes, but the decision to not invest always came down to the inability of poor farmers to access the feed grains and health products required for these enterprises (Smith et al., 2013).

It was in view of this that the African Chicken Genetic Gains (ACGG) project in Nigeria, introduced six improved tropically adapted chicken breeds (iTABs) to smallholder poultry farmers under the scavenging and semi-scavenging management systems (Yakubu et al., 2019).

Soinaya (2015) reported that the adoption of these improved tropically adapted chicken breeds (iTABs) into the tropically farming system has the potential for achieving the prospect of Mahendra (2015) and in addition, has the capacity of multiple functions which includes: fighting malnutrition, better price, consume minimum land, labor and capital, required low skilled labor as well as providing regular source of income to smallholder poultry farmers. This implies that these iTABs are characterized by huge genetic resources necessary for transforming the smallholder poultry production in the tropics (http://www.africacgg.net - June 2nd 2017). Bamidele et al. (2020) reported these iTABs to be SHP-specific hybrid germplasms with dual-purpose functions (meat and egg production), while Alabi et al. (2020) asserted that the introduction of the iTABs has reportedly improved the livelihoods, food security and socio-economic status of SHP farmers under the ACGG project in Nigeria.

However, these genetic resources (iTABs) altogether could exhibit a striking variety of characteristics as a result of genetic changes during their development under different environments and towards different selection objectives. Hence, it becomes necessary to further identify and evaluate these genetic resources in terms of biodiversity, conservation, and utilization as to their potential contribution to agricultural production in the future using molecular characterization.

Molecular characterization can play a role in uncovering the history and estimating the diversity, distinctiveness and population structures of Animal Genetic Resources (AnGR). It can also serve as an aid in the genetic management of small populations to avoid excessive inbreeding (Bamshad et al., 2003). Microsatellites have been widely and frequently used for evaluation of genetic diversity in livestock including chicken (Sunnucks, 2011). Although, they are good for pedigree and population structure analysis, however, they may not be a very reliable method for deep phylogenetic analysis because they do not offer insight into distant relationships (Laga et al., 2004).

However, mitochondrial DNA polymorphism has uncovered extensive insight into phylogenetic and genetic distance analysis. mtDNA markers may additionally offer a rapid way of detecting hybridization between farm animal species or subspecies (Nijman et al., 2003). Galtier et al. (2009) reported that mtDNA markers have been found in great abundance in the cell cytoplasm and hence easily amplified. Saccone et al. (1990) suggested that different regions of mtDNA evolve at different rates, while the displacement loop (*D-loop*) is the major control region for mtDNA expression and diversity. The evolutionary rate of the mitochondrial genome is five to 10 times higher than the nuclear genome, and has a wider range of utility in the study of ancient population structures, inter-species variability, relationships between populations or species, and identification of maternal lineages (Brown et al., 1982; Niu et al., 2002; Liu et al., 2004; 2006). The polymorphism in the sequence of the hypervariable region of the *D–loop* or control region of mtDNA have contributed greatly to the identification of the wild progenitors of domestic species, the establishment of the geographic patterns of genetic diversity and the understanding of livestock domestication (Bruford et al., 2003) and on chickens (Muchadeyi et al., 2008; Razainfindraibe et al., 2008; Adebambo et al., 2010; Nwacharo et al., 2010).

The origin and domestication of chicken are well documented. Crawford (1990) reported that recent chicken is formed from several *Gallus* sub-species and the number of sub-species involved in the origin of chicken is controversial and uncertain. The pluralism scholars suggested that the red jungle fowl *(Gallus gallus)* is the main ancestor whiles the Ceylon jungle fowl (CJF) (*G. lafayetti),* Grey junglefowl (GrJF) *(Gallus. sonneratti*) and Green jungle fowl (GJF) (*Galus. Varius)* are the secondary. In contrast to this, Darwin (1868) suggested that the red jungle fowl is the only ancestor of all domestic chicken hence suggesting single maternal origin of domestic chicken. The maternal lineage sharing has been reported among different indigenous chicken breeds of various geographical locations (Fumihito et al., 1996; West and Zhou, 1988; Liu at al., 2004; Nishibori et al., 2005). West and Zhou (1988) proposed an earlier origin in Southeast Asia, before the 6000 BC, based on archaeological evidence from China, Southeast Asia, and Europe, and palaeo climatic evidence in China. Liu et al. (2006) supported the theory of multiple origins in South and Southeast Asia from China and/or surrounding areas (i.e., Vietnam, Burma, and Thailand), and the Indian subcontinent for Asian jungle chickens. Additionally, Oka et al. (2007) confirmed the originality of Southeast Asian chicken, thus, suggesting that Japanese native chickens have multiple origins. Bjørnstad et al. (2009) suggested single maternal origins for the southwestern Nigerian domestic chicken while Cuc et al. (2011) reported multiple maternal lineages of Vietnamese local chickens.

According to suggestion based on a whole mitochondrial genome study, insight into the phylogenetic analysis of the origin of domestic chicken reported 14-haplogroup setting (A-J and W-Z) based on mtDNA D-loop sequences in 206 red jungle fowls from eight countries (China, India, Indonesia, Laos, Myanmar, Philippines, Thailand, and Vietnam) and 3797 indigenous chickens from 30 countries in Asia, Europe, Africa, South America, and six Pacific islands (Miao et al., 2013). These haplogroup setting are otherwise referred to as clades.

However, insight on variation across the entire chicken mitochondrial genome can be useful for defining the molecular basis of many metabolic disorders, diseases, and abnormalities that affect chickens (Guan et al., 2012). Thus, genetic diversity within and between species in a given population serves as an important tool required to withstand any environmental changes for adaptation and survival (Abde-Basset et al., 2014). Increased population genetic diversity is highly correlated with increased population fitness; hence maintenance of genetic diversity is imperative for conservation.

Therefore for the conservation of the iTABs, for effective recommendations for a planned breeding program aimed at improving and continual distribution of these breeds to smallholder poultry farmers in Nigeria specifically in the South East, we evaluated the genetic diversity of the iTABs introduced to SHP farmers in Imo State Nigeria using the hyper variable D-loop region of mtDNA in order to obtain the level of mitochondrial DNA polymorphism among the populations, to estimate the genetic distance

and gene flow (diversity) within and among the chicken populations sampled, to establish pylogenetic relationships among the iTABs and to ascertain the maternal lineage and origin of the breeds. For the context of this study, we only analyzed haplogroups C, D E F, H, I and J.

Materials and Methods

Study Area and Populations

Samples were collected from three on-farm sites of the African Chicken Genetic Gains (ACGG) project in Imo State, Nigeria. The ACGG is a platform for testing, delivering, and continuously improving tropically-adapted chickens for productivity growth in 3 selected African countries: Ethiopia, Tanzania and Nigeria (www.africacgg.net). In Nigeria, the on-farm test was conducted from 2016 – 2018 in which six selected improved tropically adapted chicken breeds (iTABs) that originated in various localities of Nigeria, France and India; were distributed in five zones represented by 'States' inclusive of Imo State (Fig. 1). Imo State is located in the humid forest agro-ecological zone of southeastern Nigeria, and lies between Latitude 4º45' N and 7º 15'N; Longitude 6º50' E and 7º25'E. This agro-ecological zone is characterized annually, by an average rainfall of 2219mm, average relative humidity of 80.0%, and a temperature (^{0}C) range of 26.4 (min) – 32.1 (max) (Yakubu et al., 2019).

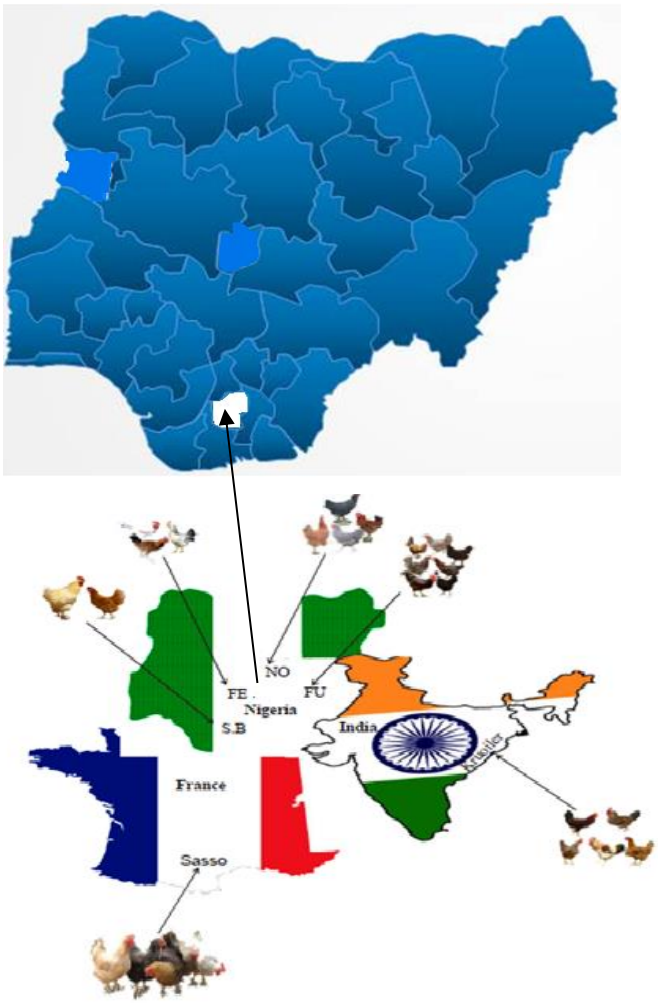

Figure 1. Map of Nigeria showing the study location of Imo State and the breed origin of the iTABs developed by the ACGG project team (samples used). White space in the map represent the study location-Imo State.

Experimental Birds/Sample Collection

A total of 77 chickens from the six iTABs (i.e., 12 each of FUNAAB Alpha and Noiler, 13 each of Fulani, Kuroiler, and Sasso and 14 Shika Brown) were randomly selected from six households in each of the three project sites of ACGG in Imo State. (Fig, 2 and Table 1). The chickens were raised under the semi-scavenging and scavenging management systems. The hens were sampled at the peak of laying while the males were sampled at an average body weight of 2kg. 2ml of whole blood were collected from the wing vein of each sampled chicken into ethylene-diamine-tetra-acetic acid (EDTA) tubes and stored at –20ºC prior to laboratory analysis.

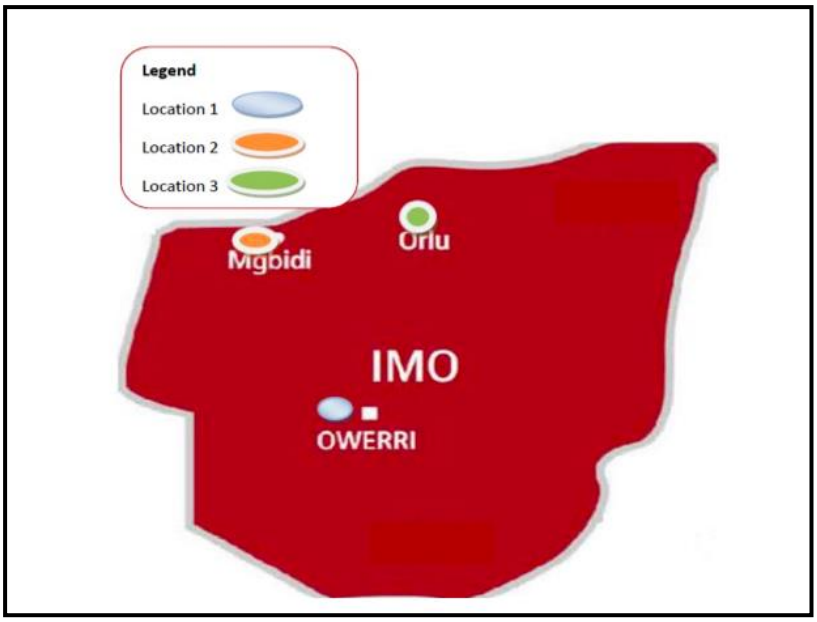

Figure 2. Map of Imo State showing the three study locations.

DNA Extraction, Polymerase Chain Reaction (PCR) and Sequencing of D-Loop (Control) Region Of mtDNA

DNA was extracted from 200 µl of whole blood samples with the aid of Quick-DNATM Mini prep Plus Kit (Zymo Research, USA). All procedures were carried out according to the manufacturer's recommendations. A five hundred and ninety-two (592) base pair region of the mtDNA D-loop was amplified by polymerase chain reaction (PCR) using L16750:5'- AGGACTACGGCTTGAAAAGC -3' (Desjardins & Morais, 1990) (for the forward primer, and H547: 3'- ATGTGCCTGACCGAGGAACCAG -5', (Komiyama et al., 2003) (for the reverse primer, as suggested by (Mobegi et al., 2005). The amplification was done with a Gene Amp PCR System 9700 (USA). Polymerase chain reaction was conducted at 96ºC for 15 mins. The second step involves 35 cycles consisting of 30-sec denaturation at 95ºC, 30 sec annealing at 56ºC and 30 mins extension at 70ºC, with a final extension at 70ºC for 5 mins. Polymerase chain reaction products were electrophoresed at 120V in 20 mins on 1.5% agarose gels (Fig. 3), and purified before sequencing using exofast protocol following the manufacturer's guide. Four hundred and fifty base pairs (450bp) D-loop region of the mtDNA was sequenced at STAB-VIDA laboratory Quinta De-forre Portugal using G16750 x L sequencer with 20 µl reaction comprising at least 20ng of purified PCR product as template DNA, 8 µL of Big Dye Terminator Reaction Mix (dNTPs, ddNTPs, buffer, enzyme and MgCl2), 8 µl of deionized water, 2 µl of primer programmed at 35 cycles at 95°C for 10 seconds, 60°C for four minutes. Only the forward primer (5'- AGGACTACGGCTTGAAAAGC -3') was used for the sequencing

Statistical analysis

Finch TV software version 1.4.0 (www.geospiza.com/fintchtv) was used to view, assemble and edit the sequences while a total of 298 bp were realized and used for subsequent analysis. MEGA version X

(Kumar et al., 2018) was used to align the D-loop sequence to the *Gallus gallus* reference sequence (Accession No: AB526207), following 1000 bootstrap replicates, a maximum Likelihood (ML) tree was generated. Nine reference sequence from the most frequent haplotypes of Liu's network (Liu et al., 2006) and the three additional clades (D, G and F) of Oka et al. (2007) were included in the analysis (Table 2). Network 10.2.0.0 (Available at https://www.fluxus-engineering.com) was used for the median-joining of both the phylogenetic tree of the haplotypes of the Improved Tropically Adapted Chicken Breeds (iTABs) used in this study and also for estimating the extent of relationship between the present studied iTABs with the D-loop sequence of other chicken (Liu et al., 2006 and Oka et al., 2007) obtained from GenBank. DnaSP 6.11.01 software (Rozas et al., 2017) was used to estimate diversity indices based on DNA polymorphism in the aligned regions including haplotype diversity (Hd), level of genetic distance, and FST (Wright and Pickton, 1998); Arlequin 3.5.2.2 software (Excoffier and Lischer, 2015) was used to estimate hierarchical analysis of molecular variance (AMOVA) based on distance method (Pairwise differences) (Wright and Pickton, 1998; Excoffier et al., 1992; 2006).

Table 1. Study samples and descriptions

Breeds (abbreviation)	**Sample size (Population size)**	**Longitude and Latitude**	**Main skin color**	**Main shank color**	**Main beak color**	**Centre of breed developm ent**
Shika Brown (SB)	14(210)	Lat 4º45'N and 7º15'N Long 6º50'E and 7º25'E	Brown	Golden-brown	Brown	Nigeria
Fulani (Fi)	13(66)	Lat 4º45'N and 7º15'N Long 6º50'E and 7º25'E	Spotted white	Brown	Grey	Nigeria
Sasso (Sa)	13(210)	Lat 4º45'N and 7º15'N Long 6º50'E and 7º25'E	Spotted white	Light brown	Grey	France
Kuroiler (Ku)	13(175)	Lat 4º45'N and 7º15'N Long 6º50'E and 7º25'E	Spotted grey/ Black	Brown	Grey	India
Funaab-ALPHA(FU)	12(68)	Lat 4º45'N and 7º15'N Long 6º50'E and 7º25'E	Black	Black	Black	Nigeria
Noiler (No)	12(140)	Lat 4º45'N and 7º15'N Long 6º50'E and 7º25'E	Spotted grey/ Black	White	White	Nigeria

450 bp amplicon size for the iTABS: Noiler (No), FUNAAB Alpha (Fu), Shika-Brown (Sb), Kuroiler (Ku), Sasso (Sa), and Fulani (Fi), M= 200 bp molecular –weight size marker
Numbers in parenthesis represent the population size of each breed found in Imo State. Numbers besides the parenthesis represents the number/size of each breed used for this experiment. Source (http://www.africacgg.net-2nd June, 2017; Okorie, 2015).

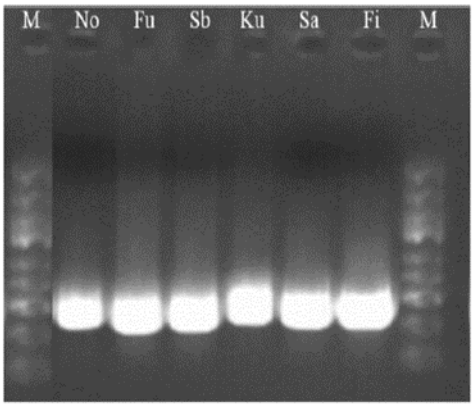

Figure 3. Polymerase chain reaction gel electrophoresis result of mtDNA D-loop region showing a
Table 2. Haplotypes of reference breed obtained from GenBank.

S/N	Haplotype Name	Accession No. in GenBank	Reference
1	Liu_A1	AB114069	Liu et al. (2006) haplotype A1
2	Liu_B1	AB007744	Liu et al. (2006) haplotype B1
3	Liu_C1	AB114070	Liu et al. (2006) haplotype C1
4	Liu_D1	AY588636	Liu et al. (2006) haplotype D1
5	Liu_E1	AB114076	Liu et al. (2006) haplotype E1
6	Liu_F1	AF512285	Liu et al. (2006) haplotype F1
7	Liu_G1	AF512288	Liu et al. (2006) haplotype G1
8	Liu_H1	D82904	Liu et al. (2006) haplotype H1
9	Liu_I1	AB009434	Liu et al. (2006) haplotype I1
10	Oka_D6	AB268535	Oka et al. (2007) haplotype D6
11	Oka_G1	AB268545	Oka et al. (2007) haplotype G1
12	Oka_F1	AB268543	Oka et al. (2007) haplotype F1

Results

We determined the haplotype sequences of the 298 bp fragments of the mtDNA D-loop region in 77 individuals from 6 populations of the Improved Tropically Adapted Chicken (iTABs). The alignment with the reference sequences produced 329 nucleotide sites, a total of 8 haplotypes, with 150 polymorphic sites (Fig. 4) consisting of 140 singleton and 10 parsimony informative sites (Supplementary Table 1). The highest haplotype had 50 individuals of the iTABs found under haplotype 2, followed by 10 individuals found under haplotype 1, and 5 individuals of iTABs found under haplotype 5. The least common haplotypes had 1 individual of the iTABs each for haplotype 3 and 4. Haplotype 5 had 5 individuals of the iTABs, haplotype 6 and 7 contained 4 individuals of iTABs each while only 2 individuals were found under haplotype 8. All variable sites were due to substitution mutations, and 94.6% of these mutations were transitions.

Genetic Diversity Indices of iTABs

Table 3 shows values for diversity indices for the study of mtDNA *D-loop* of the improved tropically adapted chicken breeds (iTABs) in Imo State Nigeria. The observed haplotype diversity among the populations was 0.558±0.063. Within the populations, varied polymorphic sites were obtained with the lowest value (*S*=1) each in Fulani and Shika Brown and the highest (*S*=11) in Noiler. The number of haplotypes in each population of iTABs ranged from the lowest value (H=2) in Fulani and Shika Brown to the highest value (H=5) in Noiler (Table 4). The overall haplotype diversity was 0.558±0.063. Haplotype diversity within populations was greatest in in FUNAAB Alpha (0.742±0.084) and lowest in Fulani (0.154±0.126).

Also, the overall nucleotide diversity among the six populations was 0.0064±0.0013; while within the populations, nucleotide diversity ranged from 0.0005±0.0004 in Fulani to 0.0106± 0.0024 in FUNAAB Alpha. Within the populations, Tajima'D though negative (-1.942) but showed significant value at

(P<0.05) in Noiler; and ranges from the least 0.140 in Noiler to the highest 1.176 in Kuroiler. Among the population of study, Tajima'D revealed a negative and non-significant value -1.379 at (P<0.01).

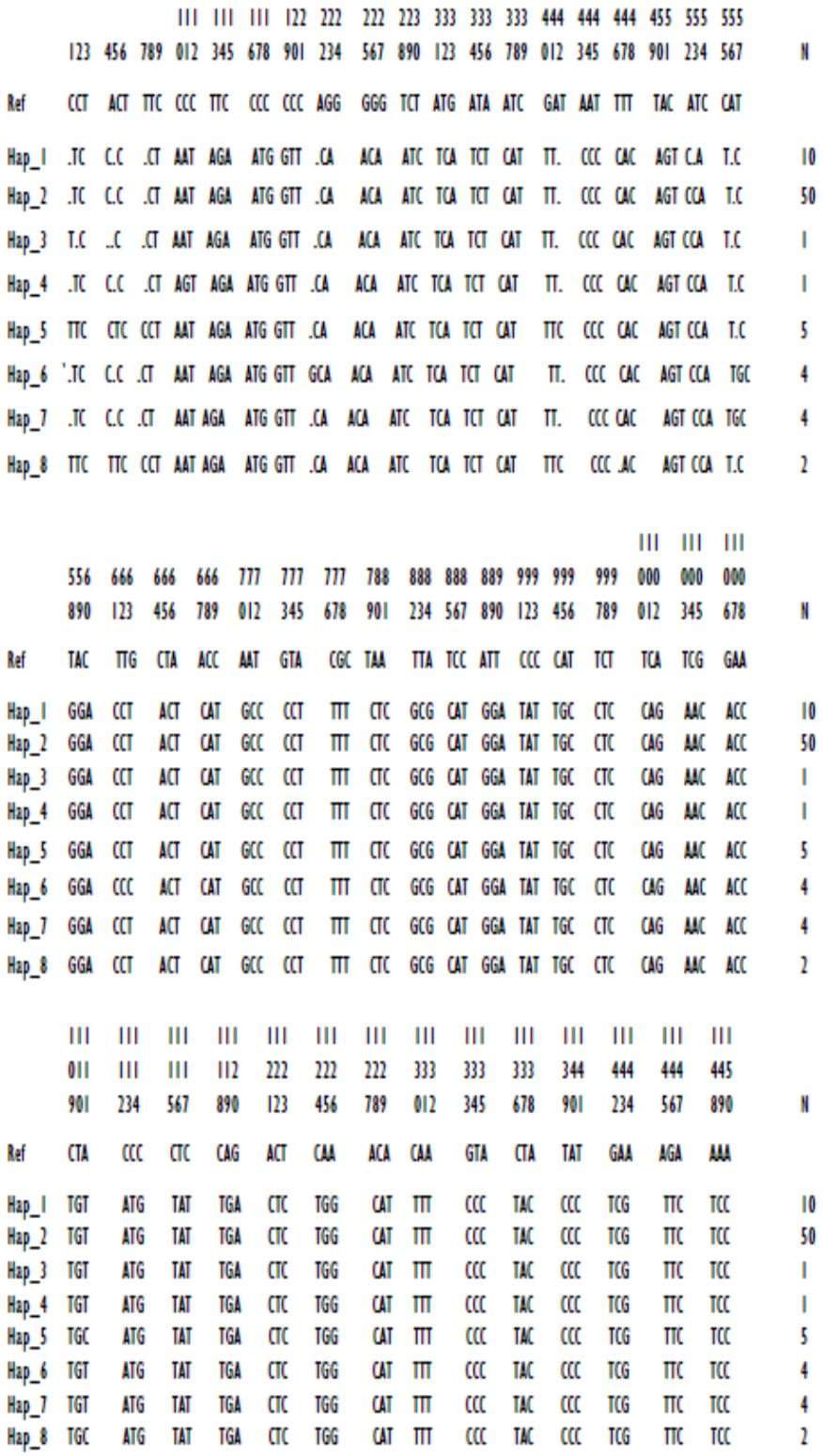

Figure 4. The polymorphic sites of 8 mtDNA *D-loop* haplotypes of six improved tropically adapted chicken breed and their frequencies (N). Vertically oriented numbers indicate the site position and the sequences shown are only the variable sites. Dots (.) indicate identity with the reference sequence (Ref)

Table 3. Genetic diversity indices of iTABs in Imo State

	Among populations	Within population				
Diversity indices		Noiler	FUNAAB-Alpha -	Kuroiler	Shika Brown	Sasso	Fulani
Number of gene copies	77	12	12	13	14	13	13
Number of Variable sites (*S*)	15	11	9	8	1	7	1
Number of haplotype (h)	8	5	4	4	2	3	2
Hd± SD	0.558± 0.063	0.576± 0.163	0.742± 0.084	0.603± 0.131	0.363± 0.130	0.295± 0.156	0.154± 0.126
Nd±SD	0.0064± 0.0013	0.0077± 0.0034	0.0106± 0.0024	0.0078± 0.0033	0.0012± 0.0004	0.0059± 0.0031	0.0005± 0.0004
Tajima' D	-1.379	-1.942	0.213	-0.522	0.324	-0.904	-1.149
Fu's F	-0.389	-0.062	2.110	1.343	0.643	2.035	-0.537

*iTABs= Improved Tropically Adapted Chicken Breeds; SD=Standard Deviation; Hd =Haplotype diversity; Nd= Nucleotide diversity; Tajima'D significant value within population (**P<0.05). Fu's F significant value (*P<0.05)*

Table 4. Haplotype frequencies in *D-loop* of the six Improved Tropically Adapted Chicken Breeds (iTABs) in Imo State

Haplotype	Noiler (12)	FUNAAB Alpha (12)	Shika Brown (14)	Kuroiler (13)	Sasso (13)	Fulani (13)
Hap_1	1(0.083)	1(0.083)	0	8(0.615)	0	0
Hap_2	8(0.667)	5(0.417)	11(0.786)	3(0.231)	11(0.846)	12(0.923)
Hap_3	1(0.083)	0	0	0	0	0
Hap_4	1(0.083)	0	0	0	0	0
Hap_5	1(0.083)	2(0.167)	0	1(0.083)	1(0.083)	0
Hap_6	0	4(0.333)	0	0	0	0
Hap_7	0	0	3(0.214)	0	0	1(0.083)
Hap_8	0	0	0	1(0.083)	1(0.083)	0

Numbers under each breed (beside the parenthesis) represent the number of such breed within the haplotype and numbers in parenthesis represent the frequency of the breeds found under the respective haplotypes.

Genetic Distance (D) among Populations

Table 5 revealed the genetic distance estimated among populations. The observed genetic distances were relatively the same across breeds with a little deviation. It ranges from (0.000±0.000) lowest between Fulani and Shika Brown which recorded zero genetic distance to 0.018±0.009 between Kuroiler and FUNAAB Alpha. Analysis of molecular variance (AMOVA) based on F-Statistics was obtained in order to understand the partitioning of the level of genetic diversity of the iTABs populations (Table 6). The result revealed that 87.89% of the total genetic variability occurred within individuals in the population of the iTABs. Also, 3.43% and 8.67% of the variations were found among groups and among populations within groups, respectively. Fixation indices (F_{ST}) observed was 0.03431 among groups, 0.08982 among populations within groups, and 0.12105 within populations.

Table 5. Genetic distance of the six iTABs in Nigeria

	No	Fu	Sb	Ku	Sa	Fi
No		0.006	0.002	0.008	0.004	0.002
Fu	0.012		0.005	0.009	0.006	0.005
Sb	0.005	0.008		0.007	0.003	0.000
Ku	0.014	0.018	0.011		0.008	0.007
Sa	0.008	0.012	0.005	0.015		0.003
Fi	0.005	0.008	0.000	0.011	0.004	

Below diagonal represents distance estimate (d); above diagonal represents Standard deviation. Analysis was computed in Bootstrap model. (No=Noiler, Fu=FUNAAB Alpha, Sb=Shika-Brown, Ku=Kuroiler, Sa=Sasso and Fi=Fulani).

Table 6. Analysis of Molecular Variance (AMOVA)

SOV	Df	Sum of Square	Variance components	%Variance	(F_{ST})	P-value
Among groups	1	2.808	0.03027Va	3.43	0.03431	0.282±0.014
Among populations within groups	4	7.020	0.07653Vb	8.67	0.08982	0.010±0.003
Within populations	71	55.056	0.77543Vc	87.89	0.12105	0.002±0.001
Total	76	64.883	0.88223			

SOV=source of variation; Df= degree of freedom; SS= sum of square; FST=Fixation indices.

Pylogenetic Relationship of the iTABs

The phylogenetic relationship of the iTABs from six different populations based on the mitochondrial *D-loop* is presented in (Fig. 5). The phylogram divided the iTABs populations into two main clusters defined by five haplogroups which separated the samples based on the evolutionary relationship. Further analysis using the median-joining network analysis of the 8 haplotypes found among the study populations (Fig. 6) revealed three major Clusters (Cluster A, B, and C). Cluster A centered on haplotype 2 which comprised of the majority of the haplotypes (haplotypes 4, 1, 7 and 6) found in this study. Cluster B comprised of only haplotype 3 and was obtained after 4 mutations from Cluster A. Lastly, cluster C comprised of haplotype 5 and 8 with 4 mutational distances.

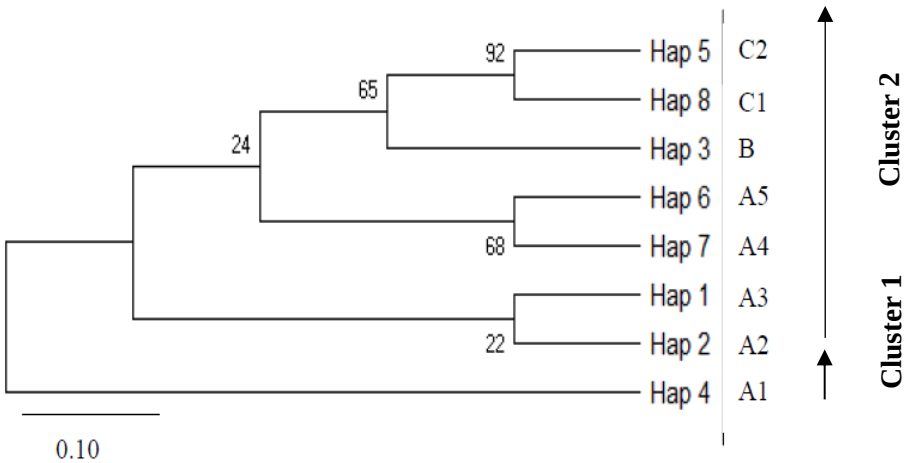

Figure 5. Maximum Likelihood Phylogenetic tree showing the evolutionary relationship between the six populations of iTABs haplotypes based on mitochondrial *D-loop* region. The numbers at nodes represent the percentage bootstrap values for interior branches after 1000 replications.

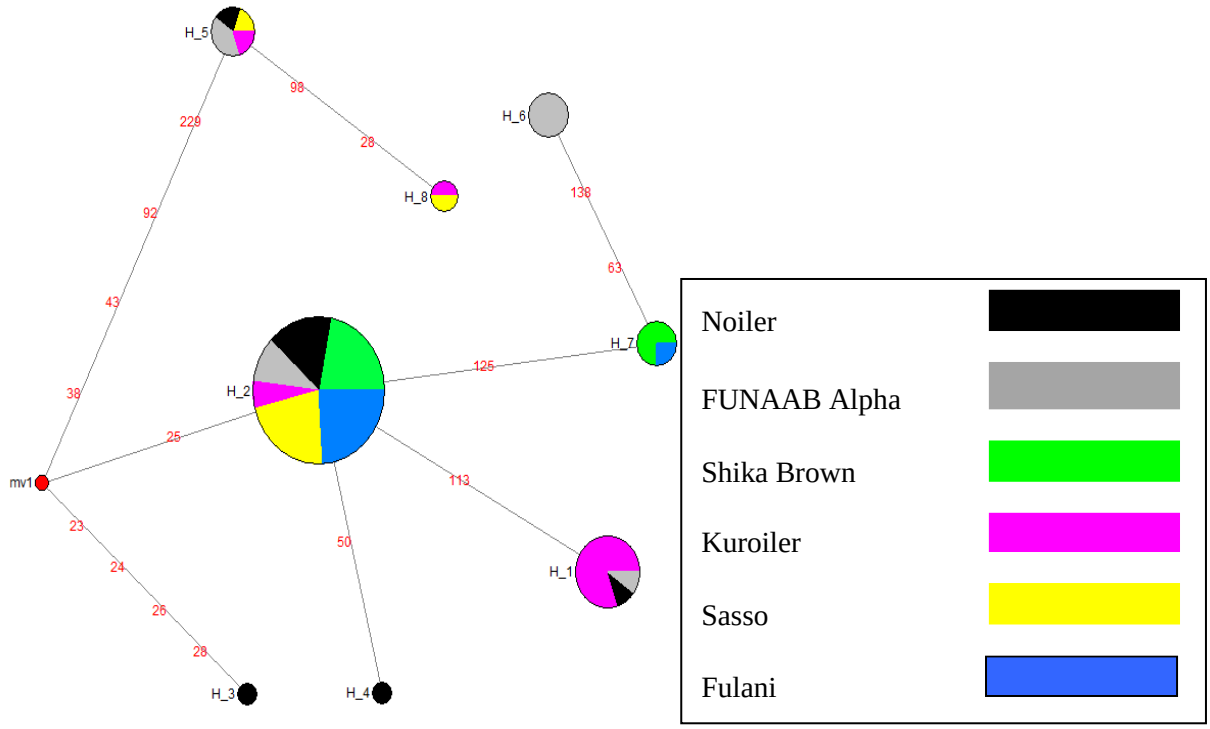

Figure 6. Median joining network profile of mtDNA D-loop haplotypes observed among the iTABs. The circle size corresponds to haplotype frequency and the numbers on the line correspond to mutational positions connecting haplotypes. Empty circles are median vectors used in connecting indirectly related haplotypes.

Maternal Lineage/Inheritance

In order to evaluate the maternal lineage of the iTABs, a maximum likelihood phylogenetic tree was reconstructed from 8 haplotypes identified in 77 sequences of iTABs together with 12 reference sequences of Liu et al. (2006) and Oka et al. (2007) (Fig 7). The phylogram split all the populations into two main clusters (clusters one and two). Cluster one is further divided into two sub-clusters (sub-cluster one and two). On the other hand, cluster two is further divided into two sub clusters (sub cluster three and four); while sub cluster four was splited into a new lineage with a recent common ancestor. In all five haplogroups were identified (haplogroup A, B, C, D and E), each containing four haplotypes. Two haplotypes from the studied population (haplotypes A2 and C1) and two haplotypes of LiuA1 and E1 belonged to Haplogroup A (Fig7). Also, two haplotypes of the studied iTABs (Haplotype A3 and A5) together with Liu F1 and Oka G1 are classified into haplogroup B. Similarly, haplotypes A4, C1, and A1 of the studied population belonged to haplogroup C together with Liu C1 haplotype.

Haplogroup D was shared by haplotypes of reference sequence from Liu and Oka; whereas haplogroup E contained only one haplotype (haplotype B) of the studied population together with Liu H1 and B1 and Oka F1 haplotypes. Among the five haplogroups identified in the studied populations, haplogroup A and C comprised mostly of the iTABs used in this study, which are found under A2 and A3 respectively.
The Media Joining Network profile in (Fig. 8), revealed a total of nine (9) haplotypes. The median joining network of iTABs populations clustered into five main clades presented in Figure 8. In this study, clade A, B and C are grouped as one (ABC), as there was no clear evidence of ancestral divergent. However, all the haplotypes of the iTABs populations (n=8; 100%) are in clade ABC whereas all other haplotypes of the reference sequence, were in clade D and E (grouped as DE).

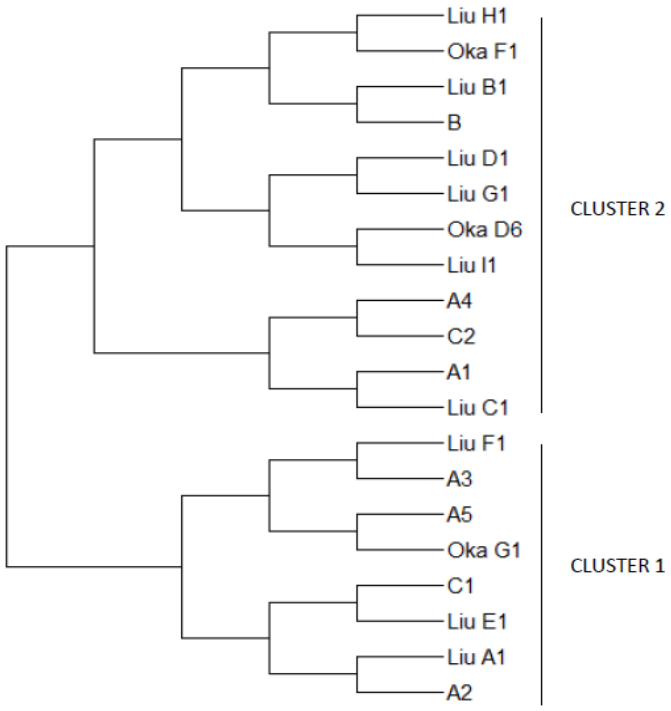

Figure 7. Ancestral states were inferred using the Maximum Likelihood method and Kimura 2-parameter model using MEGA 7.0 software. The analysis includes 8 haplotypes identified in 77 sequences of iTABs and 9 reference sequences from the most frequent haplotypes of the nine clades of Liu's network (Liu et al., 2006) and the three additional clades (D, G and F) of Oka et al. (2007).

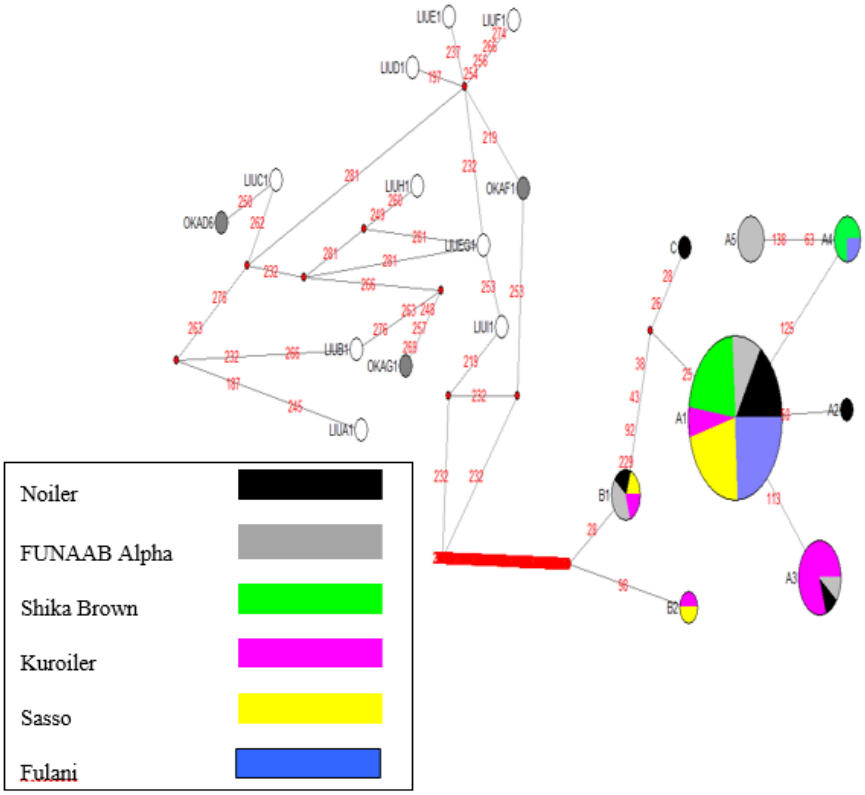

Figure 8. Media joining netork profile of mtDNA D-loop haplotypes observed in the current study. The circle sizes correspond to haplotype frequency and the numbers on the line correspond to mutational positions connecting haplotypes. Red circles are median vectors used in connecting indirectly related haplotypes.

Discussion

Genetic Diversity of iTABs in Imo State

Genetic diversity plays a vital role in the survival and adaptability of species in a given population (Frankham, 2005). Increased population genetic diversity is highly correlated with increased population fitness; hence maintenance of genetic diversity is imperative for conservation. The average mtDNA variations among the six populations as revealed by the diversity indices (Table 3) of this study indicates an average number of closely related haplotypes, and suggest that this population may have undergone a recent expansion. This was evidenced by the overall Tajima's D and Fu's Fs tests which showed negative and not statistically significant among populations thus, is consistent with a population at drift-mutation equilibrium.

The negative non-significant value for Tajima's D observed among populations could suggest that, in the presence of balanced selection between/among these chicken breeds, the evidence for population expansion is not significant. This could be attributed to an excess of rare nucleotide site variants compared to what would be expected under a neutral model of evolution. Also, the negative and non-significant Fu's Fs statistical value observed among populations indicates no strong evidence for past population expansion, and signifies the possibility of genetic hitching and background selection, also, an evolutionary force that produces a pattern similar to population expansion (Fu and Li, 1993; Fu, 1997; Okello et al., 2005). Beneficial genetic variation will generally be accumulated and maintained in the presence of rapid growth of these populations similar to (Su et al., 2001).

The average haplotype diversity value (Table 3) among population was found similar to the Algerian chicken populations (0,597) (Boudali et al,.2020) and higher than those of the African chicken populations of: Ethiopia 0.374, Sudanese 0.413 and Uganda 0.322 (Nwacharo et al., 2011); and Nigerian 0.421 (Adebambo et al., 2010). However, the values are lower than those of the African fowls of: Zimbabwe 0.730 (Muchadeyi et al., 2010) and Kenya 0.857 (Nwacharo et al., 2011).

Compared to the other iTABs, the highest number of polymorphic sites observed in Noiler reveals a higher amount of genetic variation within the population. This study also showed that Noiler had the highest number of shared haplotypes with other iTABs while FUNAAB Alpha and Kuroiler ranked second and third, respectively. This suggests that Noiler, FUNAAB Alpha and Kuroiler are more likely to have been developed from a common ancestor or could have shared ancient lineages. This is in line with the report of Torrini et al. (1993) and Ward et al. (1993) where older /more ancient populations tend to mutate longer and accumulate their mutations. Also, Research for development (2017) had it that FUNAAB Alpha chicken had undergone extensive research for improvement for more than 20 years. This result therefore indicates that Noiler, FUNAAB Alpha and Kuroiler breeds could be more genetically diverse with the haplotypes being shared with other populations. Haplotype shared observed in Shika Brown, Fulani and Sasso was very low, this suggests that these breeds must have undergone less mutational processes in their genome compared with the rest of the iTABs similar to (Stumpf, 2004).

The values of haplotype diversity obtained in this study within populations were similar to that of the Asian Vietnamese fowls (0.615 to 0.942) (Cuc et al., 2011). Therefore, these findings indicate that the mtDNA polymorphism within and among the iTAB populations raised in Imo State, Nigeria is on the average compared to most African chicken populations but similar to a few African chicken populations, and Asian chicken populations. The haplotype diversity δ is a more suitable parameter than nucleotide diversity to estimate genetic diversity in populations as it addresses the frequency of haplotypes and nucleotide differences between haplotypes. In this study, the high range of haplotype diversity observed within populations of FUNAAB Alpha, Kuroiler and Noiler indicates an existence of high molecular differences within these populations, thus suggesting that the FUNAAB Alpha, Kuroiler and Noiler are likely to have a high adaptation to environmental changes under natural selection (Steffen et al., 2008). The combination of high haplotype diversity and low nucleotide diversity, as observed in FUNAAB Alpha, Kuroiler and Noiler, can indicate a signature of a rapid demographic expansion from a small effective population size (Avise, 2000).

Genetic distance and AMOVA

Genetic distance reveals the degree of genetic differences between or within species in a population (Nei, 1987). When population has many identical alleles, the degree of genetic distance will be low and such populations are genetically more related with a common ancestor. This study revealed a high genetic distance between Kuroiler and FUNAAB Alpha and a low genetic distance between Fulani and Shika Brown. This result suggests that Fulani and Shika Brown could have been genetically bred from a common ancestor; whereas Kuroiler and FUNAAB Alpha must have had a recently divergent from a common ancestor hence they are less genetically related. Therefore, this result suggests the absence of significant genetic subdivision within Kuroiler and FUNAAB Alpha. It also implied that Kuroiler and FUNAAB Alpha has the capacity to thrive independently irrespective of the amount of gene exchange between them, as such, are more diversified than the rest of the iTABs.

Analysis of molecular variance (AMOVA) in this study revealed higher genetic variation within populations than among populations within groups which therefore suggests a high level of female mediated gene flow within population (Tserenbata et al., 2004). This implies that there is a higher maternal genetic variation within the iTAB populations. This is in contrast with Do et al. (2019) who reported higher variation among groups of Vietnamese Indigenous chicken.

Also, the positive but non-significant value for Tajima's D observed within population of Shika Brown and Fulani (Table 3) indicates that within these populations, there is a shred of evidence for high polymorphism under decreasing or low population size however; there is no significant evidence for population growth. This result is similar to the result reported by (Teinlek et al., 2018), and could be attributed to the high occurrence of balance selection on the present iTABs. On the contrary, the 3.43 percentage variation observed among groups indicates little or no population sub-structuring among populations, meaning there is little/no mtDNA variation among the iTAB populations. This result agrees with the report of Parieset et al. (2011) and indicates high maternal variations within the populations of iTAB in Imo State, Nigeria compared with that observed among groups.

Phylogenetic relationship of the iTABs in Imo State

The two main clusters identified among the populations as revealed by the phylogenetic tree, represent two ancient lineages from which the iTABs are derived. Though, this could be attributed to recent mutational event. Our finding agrees with Ohno (1997); therefore, owing to the effect of some evolutionary forces such as mutation, individuals of iTABs in both clusters were said to have shared an ancient lineage. However, due to some recent mutational events, they split into two lineages there by representing two distinct lineages from which the iTABs were derived. This, therefore, suggested that the present iTABs likely shared two common lineages originating from an ancient ancestor. Lineage I is the farthest lineage and contained only one individual of iTABs expressed in haplotype 4(A1); and is said to have maintained their ancient lineage. This result is in line with Revay et al. (2010), which therefore implies that over the years, this population probably could not have been affected by any evolutionary forces.

The five haplogroups that defined the two lineages of iTABs reveals the presence of five mitochondrial genomes among the populations. Although, some of these haplogroups had begun the process of establishing independent lineages, hence the various sub-lineages found in the tree.

The closeness of the clustering implied that mating might have occurred between lineages, thus agreeing with (Gongora et al., 2008). This is justified by the median joining network which showed the existence of two clear clusters separated by a limited number of mutational events; thus, having a recent lineage evolving with just one mutational event found in haplotype 3 (B). Therefore, we conclude that the two major clusters observed on the iTABs which centered mainly on haplotype 1 and 2, justifies the fact that the present iTABs could be derived from two common evolutionary lineages.

Maternal inheritance/origin of the Improved Tropically Adapted Chicken Breeds (iTABs)

The five haplogroups (A, B, C, D and E) identified in this study were well represented among the 14-haplogroup setting (A-J and W-Z) suggested by Miao et al. (2013). The iTABs were found under the haplogroup A, B, C and E. The two most frequently occurring haplogroups (A, and B) which contains the most frequent haplotypes (haplotype 1 and 2; represented as A3 and A2) (justified by the haplotype

distribution and the median joining network profile), were found to have shared an ancient common ancestor thus indicating the presence of a distinct clade for the studied populations. Haplogroup A and B were observed in East African Chicken population (Mwacharo et al., 2011), in South African chicken population (Mtileni et al., 2011) and in Zimbabwean village chicken population (Muchadeyi et al., 2008), but absent in other African countries. Haplogroup B distributes mainly in south central and southeast China, and southeast Asia (Miao et al., 2013).

Haplogroups C and E were also the prevailing haplogroups in the studied iTABs although in a very minor frequency. About 7.70% of the iTABs were found in haplogroup C thus indicating approximately 8% contribution of this haplogroup to the iTABs. Only 1% of the iTAB was found in haplogroup E thus indicating a very little or no considerable contribution of this haplogroup to iTABs. However, Liu et al. (2006) ; Miao et al. (2013) and Langford et al. (2013) suggested that haplogroup C has been widely distributed in East Asia, Southeast Asia and Pacific. The hplotypes of C observed in most of the iTABs were closely related to the represented haplotypes in South China, Vietnam, Laos, Sri Lanka and Japan. Haplogroup E is mainly distributed among Eurasian and South Asian domestic chickens (Liu et al., 2006 and Miao et al., 2013). The minute existence of iTABs in haplogroup E suggested no clear relationship of the iTABs with the South Asian domestic Chicken of (Liu et al., 2006 and Miao et al., 2013). The insignificant occurrence of this haplogroup observed in the studied population could be traced to the maternal lineage sharing of the iTABs (haplogroups A and B) observed in this study, with the South African Chicken of Mtileni et al. (2011). The result therefore suggests that the present iTABs were likely to be dominated by 2 maternal lineages defined in haplogroup A and B.

Haplogroup F-J and W–Z (Maio et al., 2013), were not found in the iTABs examined in the present study, which suggests that these haplogroups are either specific to other regions in Asia, or were not included in the individuals examined in the present study simply by chance. The existence of the iTABs into a distinct clade (clade II) was well represented in the seven clades identified in Asian domestic chicken (Bjornstad et al., 2013), which confirmed the existence of a contribution of single maternal lineage in all of thm. A single clade was observed in Adebambo et al. (2010); Fumihito et al. (1994; 1996). In this study, the single clade observed suggests a closer history of domestication as well as the absence of admixture between the iTABs, and the West African village chicken and southwestern Nigerian indigenous chicken.

Therefore these distinct distribution patterns of iTABs suggest that the distinct clade is likely to be descended from two common ancestors, possibly of East African origin (Mwacharo et., 2011); South African origin (Mtileni et al., 2011); Zimbabwean origin (Muchadeyi et al., 2008); south central and southeast China, and southeast Asia (Miao et al., 2013).

The results of our study suggest the possibility that iTABs chickens, East African Chicken population (Mwacharo et., 2011), South African chicken population (Mtileni et al., 2011) and Zimbabwean village chicken population (Muchadeyi et al., 2008) harbor a variety of diverse genes that regulate traits beneficial to the poultry industry, such as those which improve egg and/or meat production and quality, environmental stress tolerance, and disease resistance. Our results of mtDNA D-loop sequences suggest that there are no major genetic differences between the iTABs and subspecies of East African, South African and Zimbabwean village chicken population. The results obtained could be affected by population histories due to their differences in the mode of inheritance and mutation rates; therefore, the estimation values of genetic diversity are not necessarily positively correlated between the mtDNA D-loop sequences. To this effect whole mitochondrial genome sequences will provide a more reliable phylogenetic tree than the D-loop sequences alone. In fact, Miao et al. (2013) mentioned that *D-loop* sequencing alone could not identify differences between haplogroups Therefore, further genome-wide genetic analysis for the iTABs could aid in clarifying the origins and genomic evolution of the ITABs and West African village chickens which, in turn, would contribute to the conservation of these invaluable genetic resources.

Conclusion

The present findings identified average mtDNA variation resulting from an average mtDNA polymorphism within the population. This revealed average molecular differences within the iTABs that could result in average adaptation to environmental changes under natural selection. The total genetic variability (87.89%) clearly displayed within the iTABs population gives the indication of an average

to high level of mtDNA polymorphism. These observations are important for the process of adaptation giving the impression that these iTABs population can gradually adapt to the specific conditions in which they live. The high genetic diversity within the population could be utilized for further genetic improvement of the breeds. This result could also guide in the conservation of the local germplasm within and among these iTABs. The conservation program can utilize the ability of different genotypes to match the different environments. This would, in turn, results in sustainable utilization of the chicken products without the need to concentrate on guessing which breeds to cross for improvement on production.

Among the six populations of iTABs, Fulani and Shika Brown were more closely related and hence are not genetically differentiated. Artificial selection of the present iTABs, could be the possible cause of average genetic differentiation observed among the "slightly differentiated" populations. The result obtained in this study, also implied that Kuroiler and FUNAAB Alpha has the capacity to thrive independently irrespective of the amount of gene exchange between them, as such, are more diversified than the rest of the iTABs.

The study shows that the iTABs could have originated from two distinct maternal lineages belonging to clade A and B and are likely to be descended from two common ancestors, possibly of East African origin; South African origin; Zimbabwean origin; south central and southeast China, as well southeast Asia. Our results of mtDNA D-loop sequences suggest that there are no major genetic differences between the iTABs and subspecies of East African, South African and Zimbabwean village chicken population. The results obtained could be affected by population histories due to their differences in the mode of inheritance and mutation rates; therefore, the estimation values of genetic diversity are not necessarily positively correlated between the mtDNA D-loop sequences. To this effect whole mitochondrial genome sequences will provide a more reliable phylogenetic tree than the D-loop sequences alone.

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Author's Contributions

Okani-Onyejiaka designed and carried out the project and wrote the manuscript. **Boudali** revised the manuscript and corrected the results. **Ogundu, Oladeji, Ogbuewu** and **Aladi** contributed each to the paper write-up at different levels**; Ogundu and Ogbuewu** supervised the project, **Oladeji** supervised and directed the paper write-up while Aladi Proof-read the paper at different times. All authors discussed the results and contributed to the final manuscript.

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